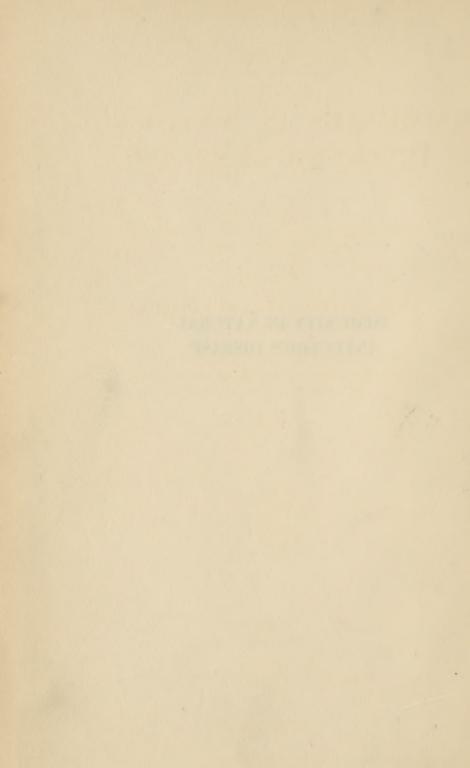# IMMUNITY IN NATURAL
INFECTIOUS DISEASE

Immunity in Natural Infectious Disease

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AUTHORIZED ENGLISH EDITION

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TRANSLATOR'S PREFACE

It is hardly to be expected that in any science which partakes of the philosophical and the speculative the phenomena there disclosed should be subject to one interpretation only. Certainly in immunology, if we consider but a few of the phenomena, such as the anaphylaxis complex, protein shock, phagocytosis, and agglutination, it is clear that varied interpretations, not only of mechanism but also of significance, have not been lacking. Doubtless we are assuming a position somewhat too secure when we affirm any single interpretation of the diverse phenomena to be the correct one—the final explanation of the means and mechanism of resistance. For surely, if the history of the subject discloses anything it shows clearly that theories of immunity are instable and must be subjected to modification as added information is gained through the application of more and more precise methods.

The earlier theories of immunity—that of exhaustion, that of retention—have been replaced by the cellular theory, and extending that, the humoral theory. New facts have necessitated new interpretations. The doctrines of Metchnikoff and of Ehrlich have dominated our ideas for a not inconsiderable period and of a certainty they have proved most useful scaffoldings upon which to build. Beyond doubt much that is contained in these theories will continue to prove useful and even with the further extension of knowledge will prove acceptable and more fully justified. Nevertheless, from many angles and from diverse sources there have appeared during recent years observations which permit justifiable question as to the value of these theories as the entire explanation of the fundamental forces of immunity. Certainly the evolution of immunologic theory need not stop here.

Among the more recent developments is the discovery of that principle, termed bacteriophagic, which indisputably possesses the property of profoundly modifying the agents against which

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immunity is primarily directed. The precise rôle of this principle in immunology is perhaps as yet incompletely defined, yet the possibilities offered are attractive and suggestive. Surely the phenomena of bacteriophagy can not be dismissed without a fair consideration. And further, these phenomena, if they play a part in resistance, must be considered in the light of our earlier knowledge.

In the French text *Les défenses de l'organisme* by Dr. d'Herelle, published in 1923, a correlation of the phenomena of bacteriophagy with those of immunology and serology was attempted. In the text here presented, containing as it does a portion of *Les défénses*, the author has gone further into the problem, and with a complete revision of the French text and the addition of several entirely new chapters he has presented a theory of the nature of immunity in natural infectious disease. Needless to say, this new concept of the mechanism of immunity involves a radical modification of some of the theoretical ideas which have hitherto dominated immunologic thought, and suggesting, as it does, so many points worthy of consideration it has seemed worth while to make this conception more readily available through a translation.

FOREWORD

The scientist, and the philosopher as well, have made many attempts at a definition of life, and apparently none have been successful, for each has selected from among the attributes of life that one, which from his own particular point of view has presented an especial interest, and he has assumed for this attribute an essential characteristic. Let us do the same. Let us define life as a power to oppose, by a specific reaction, adverse environmental conditions.

A specific reaction is not necessarily a salutary reaction for the being in which it is manifested. The reaction is not an "end" designed for the protection of the being; the organism reacts simply because a specific reaction is the expression of life itself. To a given stimulus the living being responds not by a single but by a whole series of responses, some of which tend toward the maintenance of the instable equilibrium which characterizes life, others toward its rupture. According to the preponderance of the first or the second, the being lives or dies. May one, then, speak of reactions of defense? Fundamentally, no. The specific reaction is a physico-chemical response and as such can not be an "end," although in certain circumstances, it is true, it assures the survival of the being. For man, thanks to the utilitarianists one might say, only one aspect of the question has been considered; for him the specific reaction is a reaction of "defense." Thus, despite the incorrectness of the term, it is expedient to use the word "defense," since it is well established by usage, is understood by all, and facilitates a discussion of the facts; and with the understood reservations its use does not offer any great inconvenience provided all of the finalistic idea is banished from the mind.

We will consider first the origin of the defense, that is, the nature of the physical phenomena which this defense utilizes. Exercise of defense implies necessarily the continual expenditure of energy; the living being must derive from its environment

the elements necessary to it. Moreover, the living organism should accumulate in its tissues a sufficient quantity of potential energy to enable it at any time to meet a sudden attack.

And finally, defense, from the point of view of the species, has certain interesting consequences, from which evolution in its entirety is derived. Against whom, against what, must the living being defend itself? There is not an element in the environment which may not be adverse. We will consider the general processes of defense against inanimate elements, physical and chemical. We will consider more in detail the phenomena which accompany the defense of the organism against colloids, that is to say, against those substances which present the same physico-chemical state as that of the cells of which the organism is formed.

The world abounds in living organisms, each of which must maintain its own integrity against the aggressions of other living beings in search of organic matter. Among all of these, the smallest, the bacteria, are the most formidable for their attack is insidious, and their number is such that the defense against them must be exercised at all times.

During the past forty years a special science has been developed —immunology—which is concerned primarily with the defense against bacteria. This science is still far from perfect and in many instances is based upon manifest errors which have been derived from poorly interpreted facts. These facts we will discuss. We will see that by the side of the effective defensive reactions there are others which tend to counterbalance them.

Let us repeat that the defense is not a providential act; it is accomplished by means of physical reactions which depend upon the physical properties of matter. These reactions are not to be considered as primarily directed toward the conservation of life although life results from such reactions, and it is not strange that a reactional process releases a whole series of phenomena, of which some are to be construed, from our human point of view, as acts of protection, of prophylaxis, and others as acts of contraprotection, of anaphylaxis.

Just a word as to the title of this text.

The modern mind bows only before experiment, and this is correct, but in biology experimentation is a two-edged sword,

as all too few appear to comprehend. From the moment that a finding is "experimental" some believe that it is beyond attack, that they can only accept it. But, nothing is more contrary to the truth and the hidden danger is the more insidious in that no one dreams of avoiding it.

The physicist, and the chemist as well, must always bend before the results of an experiment, for the conditions of their experiments are always "natural," Even if they wished to do so it would be impossible to invest their experiments with "artificial" conditions such as would change the results. A good experimenter should always perform good experiments, even if he lacks the logic to interpret them.

In biology, an excellent experimenter may perform very poor experiments, for the simple reason that, while for the chemist or the physicist, there is but a single experimental method, for the biologist there are two, a rational and an irrational method. The biologist selects the conditions of his experiment. He can perform an artificial experiment if the conditions which he elects are artificial, or he can conduct a true experiment if he carries it out under natural conditions. The first leads to error, the second to truth.

But alas, almost all of immunology is based upon artificial experiments, for we have conducted almost our entire study of the reactions of opposition upon laboratory animals, rabbits and guinea-pigs, and with bacteria to which these animals are *refractory*. Such a method of investigation, very good indeed for a study of the refractory state, leads only to error when applied to the study of processes of *acquired* immunity, for under these conditions only a non-existent phenomenon is being studied. An animal possessing an immunity from birth can not acquire that immunity.

Immunology, at least such as is usually designated by the name, is the study of "immunity in artificial infectious disease." Only a study of the *natural* disease, as it prevails among animals *naturally* susceptible, can lead us to a knowledge of acquired immunity. The only valid conclusions are those derived from a study of "immunity in natural infectious diseases."

Leiden, April 25, 1924.

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PART ONE
THE REACTIONS OF LIVING MATTER

CHAPTER I

The Origin of the Specific Reaction

LIFE

As yet we do not know the nature of life, we only know the properties of living matter. These properties are represented in two fundamental attributes, that of assimilation and that of adaptation. These two characteristics are necessarily always present in all living beings, and only in living beings are both to be found at the same time. When they are lost death is the consequence.

The power of assimilation consists in the property of transforming an heterogeneous substance into material like that of which the organism is formed, in proportion to the peculiar ability of the individual.

From the oxygen and the hydrogen of water, from the oxygen and the carbon of the air, and from the nitrogen carried into the soil by the rain and from the salts which are there to be found the plant builds up living vegetable matter, utilizing for this constructive work the energy provided by the light rays, of which a part is accumulated in these substances. The herbivore seizes upon this vegetable matter utilizing a portion as a source of energy to maintain itself as an animal of independent locomotion and transforming another portion into "herbivore substance" of the type of its own peculiar substance—"caterpillar substance," "chicken substance," "sheep substance," etc. The carnivore devours the herbivore, utilizing a part of the "herbivore substance" as a source of energy and transforming a portion into "carnivore substance," such as "shark substance," "swallow substance," "tiger substance," or "human substance." But a time comes when the vegetable or animal agent ceases to function through the arrest of this power of assimilation, and the animal or the vegetable dies. The bacterium then seizes upon this dead substance and makes use of a fractional portion in order

to construct "bacterial substance," as had been done before it by the herbivore and the carnivore and it likewise utilizes its energy in degrading the organic matter into its elements, returning them to the air, the water, and the soil where the plant can again make use of them. Thus the cycle is completed; all has been assimilated.

The capacity of assimilation involves as a corollary the faculty of multiplication. A bacterium, or a cell, which could assimilate without multiplication, would grow indefinitely if a physico-chemical phenomenon, to which we will return, did not intervene, causing the cell when arrived at a certain limit of size to divide into two comparable individuals.

If the conditions of the medium could persist unchanged indefinitely a being endowed with the single property of assimilation might continue to live and to perpetuate its race indefinitely. But this is not the case. The external conditions are continually varying and accordingly the organism must respond in order to live. If the variations are too sudden or too intense to permit the response to be accomplished in time the being dies. If the variation is such that conditions remain within the limits compatible with an appropriate reaction the more adaptable beings react and survive, the less apt fail to adjust themselves and succumb.

But it is not solely in response to the physical conditions of the environment that a reaction must be effected. Every living organism in quest of the energy which it can only procure from organic matter endeavors to secure it from another living being. The most abundant and the most greedy, the bacteria, to obtain the organic matter indispensible to their development, attack not only such plants and animals as may be dead, but also organisms which are alive, still capable of reacting to the end of maintaining an existence. The living being reacts without cessation, it undergoes a continuous process of adaptation. An organism endowed with but the single power of assimilation would not be viable, it could not become the origin of a race for it would be overcome immediately after birth.

The power of adaptation is the property of reacting "specifically," that is, of opposing to an excitation a reaction in which the rhythm corresponds specifically with the excitation under-

gone. For example, man is attacked by a diphtheria bacillus which secretes a special toxin. To this excitation the man reacts specifically by elaborating within his body an antitoxin neutralizing diphtheria toxin, and diphtheria toxin only. All other toxins are unaffected, for the response is adapted to the nature of the stimulation.

But every living being is subjected throughout the course of its existence to external conditions which vary continually; it is attacked by various bacteria, and it continues to live only because to each change, to each attack, it is able to react specifically. Adaptation takes place. Furthermore, each of these successive adaptations leaves its imprint within the substance of the being. Through each of these attacks the organism acquires a special "character," and living matter retains, for a greater or less length of time, the mark of the reaction effected.

A man who becomes adapted to life in a northern region acquires as a particular characteristic, the capacity to resist cold. A man who has survived the attack of a plague bacillus acquires thereby a specific character of resistance which is not possessed by the man who has passed his life in a district free of plague. The long series of successive adaptations undergone by a given individual differs from the series of every other being of the same species, in other words, each individual possesses a sum total of acquired characteristics which is peculiar to him alone. But the infinite number of characters acquired by a being in the course of his existence are transmitted inequally to his descendants. The character of "resistance," or its opposite "predisposition," to different infectious agents is a particularly apt example, and we will consider numerous examples of this type throughout the course of these pages.

Everyone possesses, then, from his very birth, a special composite of inherited characteristics, differing from the group of characters appertaining to every other individual of the same species. Moreover, throughout his life he is adding an infinite number of other characteristics forming a group which is specific to him. Thus the power of adaptation carries as a corollary, variability. Even within a single species there cannot exist two individuals which are in their entirety identical.

Such are the characteristics of living matter, assimilation and adaptation, with the properties consequent upon them, the capacity for reproduction and for variability. But it is the peculiarity of man not to be content with the things which daily observation reveals to him, he must go deeper and must know the source of these facts which he has observed. He asks from what phenomena are these properties of living matter derived. Two hypotheses are possible. Life is derived from a cellular organization, and this is the hypothesis actually adopted in a quite general fashion by biologists. But there is a second one, namely, that life, that is to say, the group of properties of assimilation and of adaptation, may be a property dependent upon a particular physico-chemical constitution of matter. Which of these should we choose? Let us consider the facts; they will provide the answer.

THE POSTULATES OF BIOLOGY

At the basis of all science are a certain number of postulates, that is, facts derived from logic but incapable of demonstration, and in this respect even the mathematical sciences are not exempt. The fundamental postulate of geometry—a straight line is the shortest distance between two points—is well known. This postulate the mathematicians tell us is not demonstrable, and from their point of view this may be correct, but for us, as biologists if we consider a fact as evident we regard it as demonstrable, for it is *experimentally* demonstrable. In reality, however, is this not a little deceit on the part of the mathematicians? Their science may be indeed pure, it may have nothing dependent upon vulgar experience where the perception of the senses is involved, but such things can not be displeasing to them, as is evident since, although only fallible experience has shown that the straight line is the shortest distance between two points, they accept it as a basis of geometry. Geometry, like all science, is in reality based upon experience. But geometry is, despite this original fault, an example of the ideal science. Assuming as a basis a certain number of experimentally demonstrable postulates, it has built up a solid structure and the centuries pass by without requiring the replacement of a single stone.

What a contrast to our poor biology! Thousands of facts have been accumulated throughout the ages, each epoch has desired to build the structure through cementing together the facts into theories, and then, after a few years some new fact has necessitated the breaking down of a portion of the wall so painfully raised only to reassemble the stones according to another plan, which in turn shortly finds itself defective by virtue of the discovery of another new fact which must be introduced into the structure. Wherein does the fault lie? Always the same; the theory which has attempted to join the facts together has not taken into account the facts themselves. The edifice has not been constructed perpendicularly, consequently its fall was inevitable. Of this we will see examples in the pages following.

What is in truth the fundamental postulate of biology? Let us turn to a recent work.

Modern biology has been for a long time dominated by two fundamental theories, the cellular doctrine and the doctrine of protoplasm which form today but a single theory, that of cellular protoplasm. Protoplasm forms the essential part of all living cells, and is found indeed only in the form of cells, isolated or united in organisms. Protoplasm is found on the earth always constituted and differentiated, chemically and morphologically, not only in the innumerable species of unicellular organisms but also in the cellular types of higher organisms.[1]

Such is the unanimous opinion, and rare are those who have ventured to contradict it, who have dared, as has Beijerinck, to separate the idea of life from the cellular concept. And yet it is but a naïve theory which can be summarized thus: Under the microscope all of that which is living is found in the form of cells, therefore all living beings are cellular and life is impossible outside of a cellular organization. To reason in this manner is equivalent to stating that the microscope is the instrument which defines life and all that is too minute to be observed with this piece of mechanism must of necessity be inert. This is the theory which, in reality, is imposed upon us by histologists, that

[1] Botazzi: Das Cytoplasma und die Körpersäfte. Translation: Verne, Le protoplasma cellulaire, système colloïdal; Masson & Cie., Paris, 1923.

is, by those students who scrutinize dead matter only, who never observe life. It would seem that only those who study the living being would be qualified to recognize life.

Who would dare, unconditionally, to maintain that the limit of microscopic visibility is precisely the limit of life? As Malle-branche stated, some two hundred years ago, "the minute animals do not have need of the microscope as much as the microscope needs the little animals."[2]

Physicists also had established a limit of the divisibility of matter. The atom was indivisible, by definition indeed, but they have found it necessary to modify this conception, for the atom, the indivisible, is a world, a diminutive planetary system, and no longer would any physicist dare to affirm that the electron itself may not be a frightfully complicated system.

This experience of the physicist ought to provide a lesson for biologists. No one can with assurance limit life without knowing what life is.

For many years we have spoken of ultraviruses and we will see that these beings can only be constituted of a simple colloidal micella. But these beings are necessarily alive for they possess all of the characteristics of living organisms; those of assimilation, of adaptation, of multiplication, and of variability. To state that a substance which possesses all of the characteristics of living beings, even those of the most highly organized, can not be a living being is incomprehensible nonsense.

A being composed of a micella can not be a cellular being, and as a consequence the cellular concept of life is necessarily false for it fails to take into consideration the facts of the case. It is thus necessary to revise the theory to provide an hypothesis which conforms to the facts, that is, that life—the sum total of the powers of assimilation and of adaptation, is the result, not of a cellular organization, but of a special physico-chemical constitution of matter.

LIVING MATTER

Experiment shows that life is always associated with protein matter. Hence, the proposition can be extended to this, that

[2] Mallebranche. La recherche de la Vérité (1674).

life is the result of a peculiar physical-chemical constitution of protein matter. As to this constitution, what can chemistry tell us?

For some fifty years chemists have concluded, from the results of elementary analyses, that the albumins are composed of simpler molecules, composed of atoms of oxygen, of hydrogen, of carbon, and of nitrogen. But they very quickly perceived that they had disregarded another element, sulfur, which they at first had considered an impurity. Thereupon a second and much more complicated formula was devised into which they introduced an atom of sulfur. But it is not sulfur alone which is always present in the albumin, for the perfecting of analytical methods revealed the presence of other metallic substances, present in different proportions in accordance with the nature of the albumin analyzed. Finally, they have desisted from composing formulae, for with the number of atoms which it would be necessary to introduce into such a molecule it would become visible to the naked eye. But this is not the only difficulty. One molecule is always identical with another molecule of the same body; all of the molecules of sodium chloride are alike, composed of one atom of sodium and one atom of chlorine. It is impossible to combine an atom of chlorine with an indeterminate number of sodium atoms. But for the albumins the situation is quite the reverse for all of the albumins are different.[3]

The assumed molecule of albumin can be decomposed into its constituent amino acids, and each albumin, in accord with the living being from which it is derived, contains a different mosaic of these acid amines. Each animal species, each individual it might be said, possesses special proteins as can be demonstrated by serological reactions.

What must we conclude? Simply that the protein molecule does not exist. Indeed, the great German chemist Fischer some

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[3] The extreme variations of the elements of albuminous substances are:

Carbon	50.6:54.5 per cent; or 1:1.08
Hydrogen	6.5: 7.3 per cent; or 1:1.12
Nitrogen	15.0:17.6 per cent; or 1:1.17
Oxygen	21.5:23.5 per cent; or 1:1.09
Sulfur	0.3: 2.2 per cent; or 1:7.33

time ago advanced the hypothesis that the albuminous materials which we subject to analysis can not be constituted of chemical substances, in the true sense of the word, but of compounds of more simple nature assembled together.

The smallest possible particle of protein matter is in reality a micella, formed of an assemblage of polymerized molecules of amino acids, and that which chemists subject to elementary analysis are the residues of the micellae.

We will return to the question of this micella; the smallest possible particle of protein matter, and consequently the smallest possible particle of living substance. The secret of life, knowledge of the physico-chemical property which confers upon protein matter the powers of assimilation and of adaptation, is still far from being known, but we do know where the secret resides— and that is indeed something. It resides in the micella.

CRYSTALLOIDS AND COLLOIDS

For a long time chemists have appreciated the existence of substances sometimes mineral but more frequently organic, whose nature disturbed them. These bodies could not be subjected to the classical method of purification by crystallization for they were always amorphous and non-volatile and among them there were not the sharp reactions, in accordance with the laws of definite proportions, which serve to establish "laws." Moreover, they left them carefully aside, and, when in the course of chemical manipulation such substances appeared, they were received with bad grace for they always brought difficulties; they passed through the filters, they became admixed with the reacting bodies. They refused, in brief, to follow the established rules. The scientist does not violate a general rule to which there are but few exceptions. He is a conservative; each thing which does not conform to the "laws" which have been evolved is a defect, a new idea, a thing which is revolutionary and which must be proscribed.

The colloidal state was first noted in 1846 by Salmi, but the first study of the colloidal condition was made by Graham in 1859. Taking a glass tube he stretched over one end a parchment membrane, obtaining thus a tight vessel. He had, in effect, a

cylinder of which the bottom was a membrane. By introducing solutions of different substances in succession into the vessel and immersing it in a container of pure water he perceived that certain of the substances passed through the membrane appearing in the water outside, while others did not. The substances which had the property of passing through the membrane, of dialyzing, he called crystalloids; those which did not pass he termed colloids. He observed that the majority of inorganic substances were crystalloids, and that, in general, the colloids were organic in nature. And, since it is true that all living matter is of colloidal nature, all of the reactions of this matter are of necessity derived from the properties peculiar to the colloidal state and it is impossible to speak of and interpret the reactions of living matter without bringing in certain physicochemical ideas.

Colloidal chemistry is a science which is even yet in its very inception; facts are being accumulated, theories are being constructed, and especially, these theories are being discussed. Scientists who are engaged in studying these problems are divided into two more or less defined groups; that is, for some the phenomena are primarily chemical, for others they are physical in nature. But to give the benefit of the doubt to all the phenomena are assuredly physical-chemical in nature. Moreover, chemistry becomes inseparable from physics, for all of the properties of substances, all chemical phenomena, are based upon physical properties as becomes more and more evident as we penetrate further into an understanding of the nature of matter. The biologist does not, however, enter into these discussions for to him it is sufficient to recognize the facts which allow him to understand the reactions of the living material which he studies. The different sciences form an inseparable whole, for it is only by knowledge of the nature of crude inorganic matter that it is possible to reach an understanding of living matter, for life is not the result of "vital reactions" but of physical reactions, which are essentially the same whether the substance be inert or living.

CONDITIONS OF THE COLLOIDAL STATE

The term "colloid" is continually being used, whereas, properly speaking, a colloid does not exist. It is the colloidal state which

exists and which is assumed by substances under certain peculiar
conditions.   In biology the colloidal state always results from the
relative conditions of a liquid, water, and of a substance insoluble
in this liquid.   Let us consider some of the conditions.

Water

Let us take a vessel filled with water.   The water is formed
of molecules of H_2O, containing therefore two atoms of hydrogen
and one atom of oxygen.[4]   Even at ordinary temperatures the

[4] An atom of any substance whatever is formed of a "planetary sys-
tem," involving an equal number of *nucleons*, elementary particles of
positive electricity, and of *electrons*, elementary particles of negative
electricity.   Whatever may be the substance—hydrogen, arsenic, or
platinum—the nucleons and the electrons which compose the atom are
identical and interchangeable.   Matter is materialized electricity.   The
elementary positive charge of a nucleon is equal in intensity to the ele-
mentary negative charge of an electron, hence the union of a nucleon and
an electron (thus forming the most simple atom, hydrogen) gives a sub-
stance electrically neutral.

In an atom, the electrical particles are arranged in a nucleus which as-
sembles all of the nucleons (whose number represents the atomic weight
of the substance) and half as many electrons.   The remainder of the
electrons (the number being equal to the numerical order of the substance
in the classification of Mendéléeff) are arranged in one or several orbits
and gravitate about the nucleus.   The external orbit, which consists of
from one to eight electrons, according to the substance under considera-
tion, is not stable.   It may lose or it may add electrons.   It is clear then
that the atom is not electrically neutral, for the excess of the electrical
charge of the nucleus is not exactly counterbalanced by the total charge
of the electrons.

If the external orbit loses one, two, three, or four electrons, that is to
say, one, two, three, or four elementary negative charges, the system as
a whole becomes electropositive, and is then a mono-, a bi-, tri-, or quad-
rivalent ion.   If, on the contrary, the external orbit completes itself
by the seizure of one, two, three, or four electrons, the system becomes
a mono-, bi-, tri-, or quadrivalent negative ion.

The atom of hydrogen, for example, is formed of one nucleon and of
one electron.   The hydrogen ion is then formed of a single nucleon and
is therefore a positive monovalent ion.   In the dissociation which takes
place in water, the OH group carries away the electron of the atom of
hydrogen, hence this group, the OH ion, possesses an elementary negative
charge.

In the sodium (Na) and potassium (K) atoms, the external orbit is

molecules of the water are not intact, for a certain number are always dissociated into two fractions, the one formed of a single atom of hydrogen (H), the other of a combination of one atom of oxygen and one atom of hydrogen (OH). But these fractions present some very peculiar properties; while the atoms and the molecules taken as a whole are electrically neutral an H atom carries a positive charge (H^+) and the OH group bears a negative charge (OH^-), the two electrostatic charges of opposite sign being of equal intensity. The union of H^+ and of OH^- gives a molecule of neutral H_2O. These fractions of molecules possessing an electrical charge have been termed ions. The degree of dissociation follows a curve which rises with the temperature. How is it that these ions of contrary sign can coexist free in a liquid without entering into combination? We do not know, but simply recognize the fact, first observed by Arrhenius, and it is necessary to accept the fact even though theory as yet offers no adequate explanation.

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formed of a single electron, and losing this, these atoms become positive monovalent ions, since there has been a loss of the elementary negative charge. In the chlorine (Cl), bromine (Br) and iodine (I) atoms the external orbit is formed of seven electrons, and in adding an electron they become monovalent negative ions.

With the atoms of magnesium (Mg) and of calcium (Ca) the external orbit is formed of two electrons, losing which they become bivalent ions with two positive charges. The conditions are similar for other substances.

The ions may be simple or compound, and in the latter case they are formed by a grouping of atoms of which one has captured or has lost one of its electrons.

The charges vary, then, not progressively, but by entire quantities, per quanta. One positive bivalent ion may replace two positive monovalent ions, or neutralize two monovalent negative ions or one bivalent negative ion. In the same way, a quadrivalent ion is worth two bivalent ions, or four monovalent ions.

These facts are thus emphasized for this question of ions is of basic importance in colloidal reactions, and consequently, in the reactions of living matter. The theory of ionization is confused with that of chemical affinities, but it is the ions, not the atoms, which combine with each other to form molecules. For example, an atom of Cl and one of Na meet. The Cl atom completes its external orbit by withdrawing an electron of Na, and the negative Cl ion then combines with a positive Na ion.

If we introduce into this water an electrolyte, a salt, a dissociation of the molecules of the salt takes place in which the relative number of the dissociated molecules increases in inverse proportion to the concentration of the dissolved electrolyte. This dissociation consists in a separation of the molecule into two parts, but here one of the two parts may bear with it one, two three, or four electrons forming thus a mono-, bi-, tri-, or quadrivalent negative ion, and on the other hand the remaining part may be lacking in one, two, three, or four electrons, thus constituting a mono-, bi-, tri-, or quadrivalent positive ion. When common salt, for example, is dissolved in water a portion of the molecules of NaCl divide into Na^+ and Cl^- ions. If the solution contains but little salt all of the molecules are dissociated.

The addition of acid to a liquid increases the quantity of H^+ ions, while the introduction of an alkali augments the OH^- ions.

Without going further into the theory of ionization we will accept the fact that water is a liquid constituted of H_2O molecules, composed of ions of H and OH. If this water is not chemically pure, and it never is so in nature and consequently in biological systems, it will contain likewise positive metallic ions and basic ions.

Within a substance, gaseous, liquid, (or even solid, although this last case it is much less obvious) the molecules are continually in motion, never in repose. If we place some water in a vessel, the molecules of H_2O strike against the walls, or in other words, exercise pressure on these walls. This molecular movement is kinetic energy, and it increases with the temperature. The speed of the molecules, and consequently the activity (which is equal to one-half of the product of the mass times the square of the velocity) increases rapidly therefore with the temperature.

Relations between water and an insoluble substance

Into this water, in which these phenomena are taking place continually, introduce a cube of metal, of platinum for example, with a side equal to a square centimeter. The presence within the liquid of this cube of platinum leads to the occurrence of a series of phenomena which are produced by those just described.

1. The molecules of H_2O strike the surfaces of the cube tending to press them back in a direction opposite to the direction of the shocks, that is, toward the interior.

2. When a fluid moistens a solid surface an adhesion of the liquid takes place, a very thin film of liquid ceases, so to speak, to be an integral part of the liquid itself and forms an atmosphere about the solid. This adhesion is so strong that a glass slide, moistened with water and carefully wiped retains upon its surface a very thin layer which can not be removed by rubbing, or even by evaporation. To remove it, it is necessary to elevate the temperature of the glass slide to 500°C.

3. Quincke, and later Helmholtz, showed that contact between two bodies develops electrical charges which form upon their surfaces two layers of opposing signs, adherent to these surfaces and subsisting in equilibrium despite the attraction which urges them to the one side or the other. This double electrical layer is due to the presence of free ions, and it is the electrical sign of these ions which determines the particular electrical sign of each of these two layers.

We might consider, then, our immersed cube of platinum, as surrounded by an atmosphere of water strongly adherent to the walls. Upon these surfaces, undergoing the shocks of the liquid molecules, is moreover adherent a layer of ions, and upon the opposing face in the layer of liquid attached, there is a layer of ions of the contrary sign and of an intensity equal to that of the first.

Obviously the intensity of these diverse phenomena is related to the *surface* of the solid, for if the surface is multiplied, the points of contact with the liquid are multiplied. This is indeed very important and provides us with the key to the colloidal state.

If we divide our cube of platinum, each side having an area of 1 sq. cm. and of course having a total surface of 6 sq. cm. into smaller cubes each one having a side with a surface of 1 sq. mm., the whole surface area will be multiplied by 10 or be equal to 60 sq. cm. If we divide it into a billion little cubes of a thousandth of a millimeter, or 1 micron to a side, the total surface of all of the microscopic cubes will be 6 square meters. And if

we divide into cubes of a millionth of a millimeter to the side, or 1 millimicron, the total surface will be 6000 square meters, or about an acre and a half.

It would be indeed difficult to obtain such a state of division by sectioning a block of matter, but we are able to obtain it readily by other procedures, in the case of the metals, in particular, by the electrical pulverization method of Bredig. By passing an electric spark through the water between two points of metal very fine particles of the metal become torn away and remain in the liquid.

In proportion as the state of division increases, the effects of the phenomena which we have described exteriorize themselves, so to speak. With the original cube, the shocks of water against the surface were balanced, that is, having given a great number of shocks occurring at the same instant upon each of the faces of the cube, an arithmetical calculation of probability shows that the sum of the shocks upon each face is practically equal. But it is not the same when each face becomes of the order of 1 square micron. The number of shocks being proportionate to the surface immersed, and the surface of a cube with a side of 1 micron being 100 million times less than that of a cube with a side of 1 cm., the number of shocks taking place at the same instant will be likewise 100 million times less, and then the law of large numbers will cease to apply. In other words, the number of shocks upon one face will no longer by chance be equal to those taking place upon the opposite face. And because of the minute mass of the particle, it will be projected in the direction opposite to that of the face which receives the greatest number of shocks at a given time. The smaller the particle, the greater is the probability that the shocks will not be balanced.

But these shocks are innumerable and are continuous, with the result that when a particle has been projected to one side an unbalanced shock will take place upon another face and the particle will be returned in the other direction, and this will continue eternally. So long as the conditions do not vary, that is to say, while the particle remains immersed and while its dimensions are not augmented as a result of the adhesion of neighboring particles it will continue to move. Within certain

quartz rocks are to be found liquid inclusions often containing microscopic bubbles of gas. These bubbles are animated as the result of the shocks of the liquid molecules, and they have been in this condition since geologic time, for millions of years, for hundreds of millions of years. This is comprehensible since the molecular movement which is the cause of the condition is eternal.

The particle will be projected horizontally and vertically in all directions, and this movement has received the name of Brownian motion, from the name of the naturalist Brown who first observed it. Moreover, philosophy had predicted the state for a long time, for it is described in a perfect manner by Lucretius in his immortal work "*De natura rerum.*"

A large mass of substance in suspension in a liquid falls quickly to the bottom of the vessel by virtue of its weight. The smaller the mass the less rapid the fall. For the force which attracts varies as the mass, that is, as the cube of the diameter, while the resistance that the liquid opposes to its movement diminishes simply as the diameter. The result is, consequently, that for a given liquid the velocity of the fall of two particles is proportional to the square of their respective diameters.

In water a marble having a diameter of 1 mm. falls about 20 cm. a second, a marble with a diameter of one one-hundredth of a micron would fall a centimeter in six years. Any increase in the viscosity of the liquid retards the fall.

Such are, at least, the conditions as determined according to the formula of Stokes, in their bearing upon Brownian movement. For particles having a diameter of 1 micron, and indeed with greater reason for those smaller, to the slowness of their fall due to the viscosity of the liquid which tends to impede the fall, must be added the influence of the unbalanced molecular shocks; and the particle, projected in all directions, horizontally and vertically as well, remains indefinitely in suspension within the liquid. Even if it by any chance becomes deposited upon the bottom of the vessel it will gradually become projected into the mass of the liquid.

We should note that this first phenomenon, under the control of kinetic energy, does not develop suddenly beginning with a

given dimension but that it operates in the same way with large masses and with small. The only result is that the phenomenon becomes more and more intense in proportion as the relation $\frac{surface}{volume}$ is increased.

The second phenomenon involved is the acquisition of a liquid atmosphere. The thickness of the liquid film which adheres to the surface of a solid is the same whatever may be the diameter of the particle immersed. This film, of but a few millimicra in thickness, is practically negligible when dealing with a large particle, but it can not be disregarded when the particle has a diameter of but a few millimicra. The mass of the atmospheric liquid may then become several times greater than the mass of the particle itself. This phenomenon appears to have attracted less attention, but in certain cases it must be of very great importance, especially in connection with the properties of the gels which constitute the state of living matter.

A third phenomenon is that of the double electric layer. It occurs in the same way whatever may be the size of the immersed particle. Here again, the number of ions bound depends upon the surface, and the greater the amount of surface in proportion to the mass, the greater the number of ions, and as a consequence, the greater the electric charge in relation to the mass.

Observation shows that in a colloidal pseudo-solution, in a *sol*, to adopt the accepted expression, the particles have no tendency to unite with each other so long as the size of the particles remains below a "critical size." From the beginning an explanation for this absence of the tendency toward cohesion has been sought in the phenomenon of electrical repulsion. Indeed, it is well-known that two bodies carrying charges of the same sign repel each other, and that they are attracted if they bear charges of opposite signs. Since the magnitude of the charge of each particle in relation to the mass of the particle is in inverse ratio to the size, it follows that the smaller the particle the greater will be the repulsion and the more stable will be the sol. But such a theory neglects an important point, namely, that each particle supports, not a single charge, but a double charge of equal intensities and of contrary signs, so that the repulsion

exercised by the external charge is counterbalanced by the internal charge. The explanation of the failure of agglomeration of particles must be something else, and this explanation has been provided by Bredig.

Surface tension

Between the molecules of a liquid there exists an attraction which has received the name of cohesion. The molecules of the superficial layer are subjected to the forces of cohesion of which the resultant is a force normal to the surface and directed toward the interior of the liquid. This force is suface tension, which, naturally, is always positive for a liquid with a surface exposed to a vacuum. But when the surface of the liquid is in contact with a gas, a solid, or another liquid, the value of the surface tension is modified, for the molecules which are at the surface are not only attracted by the molecules within the liquid but also by the molecules of the foreign substance.

For a given liquid, water for example, the only liquid with which we are concerned in biology, the modification in surface tension due to the presence of a foreign body depends upon the nature of the body. According to its nature, the value of the tension may be increased, diminished, annulled, or even reversed.

Surface tension tends to diminish the surface of an isolated compressible particle, the particle assuming a form which presents the smallest surface possible, that is, a spherical form. If several particles are together, this tendency toward reduction in surface operates upon all of them, leading to an agglomeration, and this the more vigorously as the value of the surface tension is increased. On the contrary, if the surface tension is zero, the tendency toward a diminution of surface does not develop, and as a result the cohesion between the different particles does not manifest itself, and they remain isolated. If the surface tension becomes negative the inverse tendency is operative, that is, the tendency is to an increase in surface, to a fractioning of the particles, even to a dissolution of the substance.

But one of the principal factors causing change in surface tension is the presence of an electrostatic charge at the surface of contact. This charge balances the force of cohesion, and depend-

ing on the intensity of the charge the surface tension may become annulled or rendered negative.

We have seen that the number of ions forming the double electric layer is greater in comparison with the mass of the particle, when the diameter of this particle is less, and the size of the electrostatic charge being related to the number of these ions, the smaller the particle the more the electrostatic charge is increased. Beginning with and beyond a given dimension of a particle, which might be termed the critical mass of this particle, the electrostatic charge found upon its surface becomes sufficiently increased to overcome the surface tension. The force of cohesion then being zero the tendency toward the agglomeration of the particles is lacking.

We have seen that the particles are projected in all directions by the shocks of the liquid molecules, and each of these particles in motion must meet with another sooner or later, the time depending upon the number of particles in a unit volume of the liquid, being shorter as the number increases. If the dimension of the particles is greater than the critical size, they agglomerate; if the particle is sufficiently below the critical size each particle remains isolated, suspended in the liquid.

We will see later that it is very easy to cause the flocculation of a colloidal sol, in other words to induce a precipitation of the particles. By changing the conditions of the medium flocculation results, simply because in the new conditions the surface tension becomes positive. But when the conditions are changed in the opposite direction, when the surface tension is rendered negative, then because of a tendency toward an increase in surface there occurs a fragmentation. There is a dispersion.

The solid particle is a *granule*, the granule with its adherent ions and its liquid atmosphere is a micella. The atmosphere of liquid is the "perigranular liquid," and the liquid within which the micellae float is the "intermicellar liquid." All together these things constitute the colloidal system, the collection of micellae representing the solid phase; the fluid, the liquid phase of the system.

Since colloidal reactions take place under the direction of the bound or "absorbed" ions it is necessary to consider the character of the double electrical charge.

MICELLAE

If we place a suspension of micellae of platinum, that is, a sol of platinum, in an electric field, we will see that the granules approach the positive pole, the anode. Such a result appears strange at first, for we know that if we place a solution of a salt of platinum in an electric field, the metallic ion (and it would be the same for any metal) goes to the cathode, to the negative pole. The metallic ion is therefore electro-positive, while the metallic granule is electro-negative.

We have seen that the granule is surrounded by a double layer of ions of the opposite sign. One of these layers is adherent to the surface of the granule, the other is found in the liquid atmosphere. If the granule approaches the positive pole it means that it carries a negative charge. It is not, therefore, the granule which is attracted, it is the inner layer of ions adherent to the granule and which, in the case of platinum, can only be constituted of negative OH ions. The metallic micella is formed of a metallic granule to which adheres a layer of OH^- ions, while within the liquid atmosphere is found a layer of H^+ ions. In an electric field there is a dissociation of the micella, the OH^- ions going toward the positive pole and carrying the granule, while the H^+ ions proceed to the negative pole.

It is the sign of the internal layer, adherent to the granule, which determines the sign of the colloid. Platinum, like all metals, when in the colloidal state is electro-negative.

The sign of the layer of ions adherent to the granule is determined by the nature of the granule. For a granule formed of a substance which is electro-negative this layer is electro-positive; and the reverse is the case when the granule is made up of an electro-positive substance.

A third possibility, the most important from the biological point of view, is that where the granule is formed of a substance with a double function, that is to say, where it has an equal aptitude to combine with an acid or a base. Such is the case with the amino acids, with glycocoll, $(CO_2H - CH_2NH_2)$ for example, which will yield not only a copper glycolate $(Cu(CO_2 \cdot CH_2 \cdot NH_2))$, but also a nitrogenous compound $(CO_2H \cdot CH_2 \cdot NH_3 \cdot NO_3)$.

The acid function is the most marked, hence glycocoll is very weakly positive, as is, incidentally, the case with all of the other amino acids. The granule of albumin, formed of polymerized molecules of acid amines, being thus very weakly electro-positive, permits, in a neutral medium, the negative ions to form an adherent layer and the albumin is an electro-negative colloid. It will be the same in an alkaline medium. But if the medium is slowly neutralized with a dilute acid a coagulation takes place at a particular point, and an excess of acid restores the micellae into suspension, and at this time it can be shown that the micellae have changed their sign and have become electro-positive.

All colloids in which the granule is formed of a substance with a double function are termed *amphoteric*, that is, substances which are capable of changing the sign in accord with the constitution of the intermicellar liquid. In an alkaline medium the micellae absorb negative ions, in an acid medium positive ions.

Throughout this brief discussion of the colloidal state we have taken as an example the metallic micella, for it provides the most simple case. Living matter is always found in the form of micellae, but these micellae are most certainly far more complicated than is the metallic micella. The granule is no longer constituted of a simple substance but of an assemblage of diverse bodies. And thus we return to the question of the constitution of protein materials of which the smallest particle possible can not be a molecule but a micella.

Undoubtedly each protein micella is in reality a micellar system, formed by the grouping into one large micella of elementary micellae of which the granule in each is a polymerized molecule of an acid amine. By different procedures it can be shown that, by degradation, each albumin furnishes a certain number of amino acids, each species of albumin being characterized by the quality of acid amines which it contains and by the quantitative relations of the different amino acids which enter into its composition. There is much ground for thinking that within one albumin the different amino acids form the elementary micellae, the granule of each of them being constituted of a polymerized insoluble molecule, that these diverse elementary micellae group themselves in micellar systems, each system forming an albuminous

micella. This micellar system is the smallest possible particle of an albumin, forming that which up to the present time we have termed the molecule of albumin. All protein substances present an analogous constitution.

In such a system, each elementary micella possesses its liquid atmosphere, its own perigranular liquid and its adsorbed ions. The complex protein must, therefore, be considered as involving micella, the micellar system, the intramicellar liquid, the entire perigranular liquid, and the intermicellar liquid. That there is such an intramicellar liquid is certain, and the present conception allows us to understand how it can be present and to explain the peculiarities of the colloidal state of the constituents of living organisms, particularly the phenomena of imbibition.

COLLOIDAL REACTIONS

Since all living matter occurs in the colloidal state, the reactions of living matter must be governed by the special properties of this state. For, although we are not yet to the point where we can analyse them, we must recognize the principal properties of micellae in order to grasp the nature of these reactions.

All of the properties of micellae originate in their constitution which permits them to capture, that is, to adsorb, the ions present in the fluid in which they are bathed. These ions may be provided by the liquid itself; in the case of water (the only liquid phase in biology) these ions are the H^+ and the OH^- ions. But in nature water never occurs chemically pure, it is always a solution of different salts, that is, a solution of electrolytes, among which are to be found metallic ions, both positive and negative, such as the micella may adsorb and which may enter into its constitution.

It is impossible to consider a micella quite apart from the liquid in which it is bathed; there is a constant relation, an equilibrium between the micella and the liquid medium, and all changes which take place in the medium have their immediate effect upon the micellae. Medium and micellae form a whole, the medium being the liquid phase and the group of micellae the solid phase. The two phases are jointly responsible, the one to the other. They are in constant equilibrium. This idea is of fundamental importance in biology, and permits us to interpret, for the most

part, the phenomena which take place within the living being.

An experiment of Gengou is particularly well adapted to demonstrate this interdependence between micellae and medium. If one takes a sol of alumina, having large micellae, and adds to it a definite amount of citric acid a dispersion results; a fragmentation of the micellae of such extent that the sol becomes perfectly limpid. The amount of the citric acid is of the greatest importance for the curve of dispersion shows a maximum corresponding to a given quantity of acid. The size of the micellae is, therefore, related to the composition of the intermicellar liquid.

If we withdraw ions from the intermicellar liquid, as is possible by dialysis, ionic equilibrium between the free ions of the liquid and those which have been adsorbed by the granule tends to establish itself, and ions detach themselves from the micellae. But then the electric charge also diminishes, the surface tension tends to become positive, and a fusion of micellae results with the formation of larger micellae. If the conditions change no further the new equilibrium continues, but if the subtraction of ions from the intermicellar liquid continues the processes of fusion also continue, up to the point of complete coagulation.

But flocculation can be brought about by means other than the removal of ions from the intermicellar liquid. Anything which leads to a discharge of the micellae produces it, as, in the case of positive colloids, by the introduction of electrons (necessarily with elementary negative charges) through radiation with the β rays of radium. Negative colloids are discharged and flocculated by ultra-violet rays, which, liberating electrons, diminish the charges of the micellae.

Always as a result of the neutralization of electric charges negative micellae are flocculated by acids, since acidity results, in the last analysis, from a concentration in the liquid of free H^+ ions. Positive micellae, on the contrary, are flocculated by bases, that is, by OH^- ions.

As for the amphoteric colloids, as we have seen, they take a $-$ sign in an alkaline medium, and a $+$ sign in an acid medium. At the neutral point the micella is discharged, in electric equilibrium with the medium at the so-called isoelectric point. With an

amphoteric micella the fragility becomes greater as the iso-electric point is approached, and traces of salts, yielding by dissociation monovalent ions, such as would have no effect upon stability in a medium slightly acid or alkaline, at this point provoke flocculation. At the isoelectric point, coagulation may even be spontaneous.

Within the body the protein micellae are found in an alkaline medium, hence they have a negative electric sign. But this alkalinity is very slight and consequently the least variation tends to profoundly modify the colloidal state. After death, as a result of autofermentations, the tissues assume an acid reaction, and consequently the micellae change their sign. Doubtless passage through the isoelectric point corresponds with the death of the protein micella.

Finally, there is another means of accomplishing the discharge of the micellae. Experiments upon electrical osmosis show that, having given a liquid which communicates to a surface a charge of a certain sign, by the addition of polyvalent ions of the *opposite sign* it is possible to greatly diminish the charge of this surface, or even to reverse the sign. The action of the tetravalent ions is the most marked, then the trivalent, then bivalent, that is, those carrying four, three, and two elementary electrical charges. For the monovalent ions, this action manifests itself only with high concentrations.

It is seen, in effect, that in order to provoke the flocculation of a colloid it is necessary to add to the intermicellar liquid a relatively large amount of monovalent ions with a sign opposite to that of the colloid, and that the amount required is reduced as the valence is increased. In the same quantities, the action of a tetravalent ion is about one thousand times stronger than is that of a monovalent ion.

The ions which provoke the coagulation are found in the coagulum. If the adsorbed ions are removed progressively the coagulum is gradually modified up to the point where the colloid resumes its original state. When thus restored the entire amount of the ions which led to the coagulation have been withdrawn. Such a removal is not always possible, in which case the coagulation is irreversible.

Even in the case where the adsorbed ions which have caused the flocculation of the micellae are solidly fixed it is possible to bring about the substitution of ions of the same sign. Vegetable tissues, for example, fix metallic ions (K, Ca, Fe, etc.) which it is possible to remove by washing with pure water, but if this water contains a salt in solution, the metallic ions of this salt take the place of those which had been fixed. It is not a case of chemical combinations, for it is possible to effect crossed substitutions, for example, to fix first K ions, to replace them with Fe ions, and then, by means of a solution with a suitable concentration of a K salt, to replace the Fe ions with the original K ions.

Such substitutions take place upon the micellae of a sol. If the ions which take the place of those which previously had been adsorbed are of the same sign and of the same valence the equilibrium of the colloidal system remains unaltered, the only change being in the chemical nature of the adsorbed ions. But there may be a change of equilibrium, tending toward a flocculation or toward a dispersion, if the valence of the substituted ions is not equal to that of the replaced ions. We have seen, for example, that in the case of an albumin, the substitution of a trivalent citric ion by an OH ion, both negative, leads to a fragmentation, to an intense dispersion of the micellae.

Finally, two colloids react upon each other. Two colloids of the same sign can coexist in the same intermicellar liquid, but if they bear opposite signs they are mutually flocculated through a reciprocal neutralization of their charges.

The case of an amphoteric colloid acting upon a negative or positive colloid is particularly interesting. The result is the formation of micellar complexes by the fusion of a negative (or positive) micella with an amphoteric micella. These micellar complexes enjoy the stability of amphoteric colloids. If we add, for example, a sol of gelatin to a sol of platinum, a complex form of gelatin-platinum micellae are formed which are much more stable than the sol of platinum.

Temperature exerts a considerable influence upon the colloidal equilibrium, as might be predicted since we know that there is a dependence between the micellar equilibrium and the equilibrium of the intermicellar liquid. Increase in temperature causes an

increase in the kinetic energy and, what is very important, a modification of the degree of ionization.   Elevation of temperature causes, according to the nature of the micellae, either a coagulation, as is the case with an albumin, or, more rarely a dispersion, as is observed with gelatin, with agar, and with pectic jellies. The colloidal equilibrium varies continually with the temperature.

Even time itself, and the history of a colloid may have an effect. Quite aside from all ionic modification, physical or chemical, of the medium, a colloid tends toward flocculation. It is necessary, however to beware of saying with certain biologists, that this natural tendency toward flocculation is the explanation for the ageing of organisms.   This might be true for an organism removed from the influences of the medium, one in which no exchange takes place between itself and its environment, but for an organism which is necessarily in adjustment with the medium, according to the conditions of the moment, there may as well be a tendency toward dispersion as toward flocculation. An alumina sol tends naturally toward flocculation, but if citric acid is present, the reverse takes place.   The reason for growing old is something entirely different.

The history of a colloid contributes to the nature of the reactions which it undergoes. Pickering, experimenting with different sols, has shown that coagulation is more rapid with sols which have previously been congealed, and the volume of coagulum for such a sol is considerably less, the reduction reaching even 90 per cent.

HYDROPHILE AND HYDROPHOBE COLLOIDS

Ordinarily the different colloids are divided into "hydrophile" and "hydrophobe" according as the granule of the micella is massive, that is to say, formed of a mass of homogeneous material (such as the metallic colloids) or as the granule is constituted of a mixture of solid and liquid materials.   To employ a crude image, the granule of the first is a solid block, that of the second a sponge.

At first thought, it is somewhat difficult to conceive a granule formed of lacunar substance impregnated with water.   As has already been indicated, it seems that the most logical conception of such an assemblage consists in considering the lacunar granules

as a micellar system, each micella being formed by a union of elementary micellae.

To take concrete examples, recognizing that the figures given do not pretend to being exact but are simply used fo facilitate explanation. Salmine, isolated from the milt of the salmon, is one of the most simple of the albumins. Upon decomposition it yields (approximately) 63 per cent of arginine, 12 per cent of histidine, 8 per cent of lysine, and the balance is alanine and leucine. The micella of salmine would be constituted of a granule formed of 26 micromicellae—14 of arginine, 2 of lysine, 3 of histidine, 3 of alanine, and 4 of leucine. The granule of each of these micromicellae would be composed of a granule formed of insoluble polymerized molecules of the acid amine, the adsorbed ions, and an atmosphere of water. The large micella, or rather the salmine micellar system, would likewise have its atmosphere of water and its adsorbed ions. From this one can understand the presence of water in the interior of the protein micella, and in general in all so-called hydrophile colloids. It also explains the phenomena of imbibition which these colloids present.

GELS

When, by any of the means previously indicated, a flocculation of the micellae of a sol is produced this flocculation begins by a coalescence of the micellae. Two micellae come together, then they fuse into one granule yielding a micella with a double mass. Two of these new micellae come together and they in turn combine, and the process continues by successive fusions. It seems that the force of cohesion between granules is more energetic when the granule is small, for when by successive fusions a limited mass is attained, cohesion is no longer operative. It functions effectively only in the union of micellae.

The phenomena which cause flocculation by virtue of a tendency toward reduction of surface produce likewise, in the case of complex micellae (hydrophile colloids), a reduction of the micella by the expulsion of a portion of the intramicellar liquid.

Theoretically, all substances in the colloidal state, are able to present two different aspects. In the first, which we have up to this time been considering, the appearance resembles a solution,

the micellae are floating in a liquid, and the viscosity approaches that of the pure intermicellar liquid. Such is a sol. In the second, the micellae are much more crowded, their respective spheres of action are in contact or actually penetrate each other, and the viscosity is that of a jelly.

It was stated that theoretically all substances in the colloidal state may, according to conditions, present the appearance of either a sol or a gel. As a matter of fact, with electro-positive or negative colloidal sols, containing a sufficient number of micellae in a given volume, the coagulation of the mass in the form of a gel will take place, if, after having added a quantity of a salt insufficient to induce immediate coagulation, the material is left undisturbed.

At the moment when it forms a gel Brownian movement ceases.

The transformation into a gel is accompanied by a phenomenon which must be of very great importance in biology. A gel is not homogeneous. It shows a peculiar network texture comparable to that of a sponge. The meshes of the network, or, in other terms, the walls of the alveoli, are formed of strata of micellae. Between the meshes is the intermicellar liquid in which float micellae. The contents of a gel is therefore a sol.

With colloids frankly electro-positive or negative it is essential to take the greatest precautions to secure transformation into a gel. The coagulation must be made very slowly and the quantity of electrolyte added must be very exactly regulated so that it will just establish the isoelectric point, and even then it may not succeed with all colloids. Even a trace of electrolyte in excess gives a precipitate of the micellae in the form of flakes.

With colloids which are amphoteric and especially with those colloids of which the micellae, as has been suggested, can not be simple but constituted rather of a complex micellar system the situation is different. For them, the gel is stable and is always formed, either by coagulation, or by the evaporation of a sufficient quantity of the intermicellar liquid. Moreover, here the gel is reversible, that is, the micellae may be brought to dispersion again, either by the addition of fluid, or by restoring the original ionic condition.

CONSEQUENCES OF THE COLLOIDAL STATE

Of this brief statement of the colloidal state, the most significant point to bear in mind is that the colloidal state is not a fixed condition, that matter in this state undergoes continual transformations under the influence of variations of the medium. There is a continual adaptation of the micella to the conditions of the medium, all of the changes of the medium being reflected in the micella, in its constitution and in its properties.

On the other hand, even though varying continually, material in the colloidal state retains its identity. A molecule of sodium chloride is identical with every molecule of sodium chloride which exists throughout the universe. And although representing a state of equilibrium essentially variable the micella does not cease to exist.

A micella of albumin, for example, may vary in the quality of the granules, because of a difference in the proportion of the different amino acids and in the arrangement of these amino acids within the granule, in the ionic quality, because of substitutions of ions, in the electrical sign, and in the intensity of the charge, according to the reaction of the intermicellar liquid and to the concentration of ions in the medium.

Controlled by these diverse factors, each of them being variable in intensity, it is obvious that the protein micella may vary in an infinite manner, and it can be readily seen that the elements of the blood, for example, may be the same throughout the whole series of vertebrates, but that each species, each individual even, may possess a "different blood" from that of all other individuals whatsoever. This is equally true for all tissues and for all cells.

And these things which have been said of the protein micella apply also to the micellae which constitute living organisms, both plants and animals.

THE CELL

The single fact that the ultravirus, formed of one micella, is a living being since it manifests the criteria of life—the powers of assimilation and of adaptation,—demonstrates that life is not fundamentally dependent upon a cellular organization. Above

these elementary beings are to be found beings somewhat more complex, formed of a simple aggregation of micellae, but not yet cellular, since they lack a nucleus. These are the bacteria and certain of the very simple protozoa. We will return, however, to this point in the chapters devoted especially to the rudimentary organisms. Above these, the cellular structure appears, and is maintained throughout all the higher degrees of the classification, within the vegetables, uni- and pluri-cellular, and within the differentiated protozoa and the metazoa.

Throughout the vegetable series the cell is provided with a differentiated membrane, a condition which is lacking in the animal series.

The cellular structures which have up to the present been described represent nothing as it actually is during life, as the histologists themselves recognize. Such things as have been described are really artifacts produced during the process of fixation, which is the histological term for coagulation.

Under the microscope the living cell shows simply a cytoplasm, optically empty, and a nucleus, likewise without visible structure but somewhat, though only slightly, more refractile than the cytoplasm from which it can be distinguished. The granulations, with the exception of the secretory granules which are not of the protoplasmic material but which may be present within the living cell, only appear with coagulation, that is, after death.

The cytoplasm is formed of a gel, and must therefore most probably possess the alveolar structure characteristic of gels, although the microscope does not permit of its recognition. Nevertheless, observation of the predatory protozoa, the amebae for example, and also of the white blood cells, shows clearly the presence of the alveolar structure. If a grain of litmus is engulfed by an infusorian it is immediately enclosed in an alveolus and the contents of this alveolus assume an acid reaction while the remainder of the cytoplasm continues to be alkaline. When the substance is digested the alveolus disappears and the liquid contents are then absorbed by the mass of the cytoplasm. The phenomenon is the same within the leucocyte with the single difference that the contents of the alveolus remain acid for a long time. These processes show clearly that there is first a destruction of the

fine network, a dispersion of the gel in the place occupied by the alveolus. The alveolus again forms, and is filled with a sol surrounding the prey. This sol has an acid reaction, and since this acid reaction remains localized there must be a condensation of the gel at the periphery, a condensation which functions to render the membrane impermeable to H ions. This impermeability is lost when the normal alkalinity is restored by the penetration of OH ions into the liquid of the alveolus. The increase in permeability continues and molecules of amino acids and of carbohydrates, derived from the digestion of the prey diffuse into the cytoplasmic mass. We will see that an hepatic cell elaborates, simultaneously, a large number of different enzymes. These things could not be comprehended in the absence of compartment-formation within the cell, that is, without the presence of an alveolar structure.

As a result of the nature of the substances penetrating into the cell, and as a result of the transformations which these substances undergo within this cell, the protoplasmic gel oscillates continually between tendencies toward dispersion and toward coagulation. The walls of the alveoli condense or relax, disappearing momentarily at certain points only to be re-formed the next according to the conditions of the moment. At a given time the reactions of the cytoplasm are not the same throughout (as is shown by observations in protozoa) with the result that the composition of different protoplasmic micellae can not be the same in the different parts of the cytoplasm, inasmuch as a micella is always in equilibrium with the medium in which it is bathed. At a given time some parts tend toward coagulation, others toward dissemination. The network will be more condensed in certain regions, more relaxed in others. And finally, the dissemination being carried too far the network is destroyed and a cavity filled with a sol results. But the adsorbed ions are a function of the size of the micella. In a sol containing dispersed micellae the free ions will necessarily be different from those which are present in the intermicellar liquid of the gel, and since the reaction of a medium depends upon the relative proportions between the H and the OH ions, the reaction of this cavity may be different from that of the balance of the cytoplasm.

On the other hand, it is evident that not all species of cells, as a result of the circumstances in which they vegetate, are subjected to the same extremes of variation. The variations in the condition in the different parts of the cytoplasm of a leucocyte are not comparable to those in a cell of the nervous system; intense in the first, they are reduced to a minimum within the last. But great or small, variations are taking place always between the different portions of the cytoplasm. The protoplasm of every cell is continually in motion, and this motion has its origin in the constitution of matter in the colloidal state.

Determination of the chemical composition of protoplasm is an impossibility. All that analyses can do is to indicate to us the elementary materials which enter into the composition of the cell— water, salts, carbohydrates, fatty acids, amino and nucleic acids. But it is impossible to determine how these substances are distributed in the cell or what is their arrangement. Chemistry can not solve the question of the composition of protoplasm. All that we know is that protoplasm is to be found in the colloidal state; it is a gel.

A cell being a small mass of gel, possessing in part the properties of liquids, in particular, that of being subject to the laws of capillarity, to its free surface there is a contractive tension— surface tension. This surface tension undergoes variations in accord with the nature of the liquid which bathes the cells, that is, according to the composition of the aqueous solution surrounding the cells. But the cell itself is a center of energy by virtue of the reactions of which it is perpetually the seat; its polarization varies continually, producing variations in the amount of the surface tension at the surface in contact with the internal medium. The nature and the intensity of the exchanges between the cell and this medium, even the form of the cell itself, is under the control of surface tension.

In a cell both cytoplasm and nucleus can be distinguished morphologically. The cytoplasm itself can be differentiated into protoplasm, or living matter, and diverse non-living products, such as glycogen, fatty materials, and other accumulated reserve products.

There is no essential chemical difference between the protoplasm

and the nucleus.   Both contain nucleoproteins, but in the nucleus these substances are present in far greater abundance than in the peripheral portions of the cell.

THE MICELLAR CONCEPT OF LIFE

The fact that living matter is found in the colloidal state, that it is, therefore, formed by an assemblage of micellae, that these micellae enclose intermicellar liquid, showing thus that each micella is rather a micellar system than a simple micella, and on the other hand, the fact that the ultraviruses, beings unquestionably alive as will be shown in subsequent chapters of this text and which can consist of only a single micellar system, all of these things warrant the inference that even in organisms that are highly organized the cell cannot be the unit of living matter.   It must be formed by the coördinated union of living protoplasmic micellae, each of which possesses the powers of assimilation and of adaptation with their subsidiary functions, the faculties of multiplication and of variability.   In a word, each one possesses its own metabolism.   The coördination of the different protoplasmic micellae in the cell is determined by the physico-chemical conditions of the moment.

The protoplasmic micella should be to the cell what the cell is to the entire organism.   The fundamental metabolism should be micellar metabolism, cellular metabolism representing the sum total of the metabolic activities of the micellae.

A protoplasmic micella should be a micellar system formed by the union of what might be termed protomicellae.   The granule of each protomicella should be a polymerized molecule of some substance such as an acid amine, a cyclic base, a lipoid, or a carbohydrate.   The union into a micellar system of a number of protomicellae of different amino acids, of different cyclic bases, of different lipoids, and of different carbohydrates, each with its adsorbed ions and its liquid atmosphere, should form the protoplasmic micella.   The general architecture of the protoplasmic micella should be always of the same type, whether it forms by itself an ultravirus or whether it enters into the cell of a man, but they should differ from each other through the nature of the protomicellae assembled.   In view of the number of acid

amines and cyclic bases the number of possible combinations is almost infinite. The behavior of each cell physiologically and morphologically should be determined by a micellar type, possibly by two types, one being cytoplasmic and the other nuclear. The behavior of an organism morphologically and physiologically should be determined by the union of a certain number of type micellae, each micellar type corresponding to a cellular type. Each botanical or zoölogical species should be formed by a union of fundamentally specific micellar types.

It is not necessary, however, to consider a micellar type as being a uniform, rigid assemblage, uniformity being incompatible with the colloidal state since a micella is always automatically adjusted to an equilibrium with its environment. Placed under identical environmental conditions the micellae of a single type would become identical, since their variations are reversible. On the contrary, the micellae of different types would remain different even if the conditions of the environment were common to both, since here it is not simply a question of adjustment to equilibrium but an inherent difference resident in the nature of the constituents of the micellae.

One might think at first sight that this micellar concept of life only complicates the situation, that it simply moves back the difficulties by replacing the cell by even smaller cells, retreating from cells to cellules. In reality there is a fundamental difference between the two conceptions, chiefly in that which deals with questions of metabolism. In the cellular conception it is necessary to invoke the intervention of synthesizing enzymes, or "formative enzymes," the occurrence of which is unknown, and, as we will see in a later paragraph, whose existence it is impossible to conceive. In the micellar conception such enzymes have no reason for being.

The formation from an egg of a being comparable to that, or to those, which took part in the formation of the egg remains an absolute mystery in the cellular conception, for it is manifestly impossible to believe that the egg can contain all of the different cellular types characteristic of the species. Many hypotheses have been advanced to explain this transmission of characters, but as a matter of fact, they are all derived from that suggested by

Spencer. It may well be, however, that had this philosopher known of the colloidal state (which even now can hardly be comprehended) his hypothesis would have followed exactly the form that I have given.

The "gemmules" of Darwin, the "micellae" of Nageli, the "idants" of Weismann are in reality only unfortunate deformations of the "physiological unit" particles of Spencer, for the gemmules, micellae, and idants have become simply the "character carriers" for them. On the contrary in the theory of Spencer and in that which I suggest the characters are determined by the constitution of the living particle itself.

A germinative cell should be formed by the union of the type micellae which represent the individual at the time of the formation of this cell, each type micella determining by its constitution the characters of the cell, and then of the derived tissue. The characters should be, then, simply attributes of the constitution. The fusion of two germinative cells[5] should provide an egg containing a representative of each of the type protoplasmic micellae of each of the two ancestors. Every group of two micellae of the same type, paternal and maternal, possessing the power of assimilation, should divide into micellae which should become arranged in cells, the cells in layers, the layers in tissues, and the tissues in organs. In a word, the egg should contain always at least one example (parthenogenesis), usually two examples, of each of the type micellae which enter into the constitution of the individual adult. The old adage, all cells are derived from a like cell, should be replaced by all living micellae are derived from the division into two identical parts of an antecedent micella.

Inasmuch as a protoplasmic micella can be derived only from the division of a previous micella one can conceive the possibility of the constancy of the specific composition of each living being. Variation can as readily be conceived, for the protoplasmic micella being endowed with the power of adaptation—a property possessed by even the rudimentary non-living micella— each micella should be able to be the origin of a divergent line of micellae.

[5] The spermatozoon and the ovum form, in effect, a single antigen, and, as is more important from the point of view of the present discussion, an ovotoxic serum is not specific (Girardi and Sivori).

The characters of the species should be a consequence of the union of micellar types; the characters of the individual, of reversible variations in micellae of a given type.

The reader may feel at first that this subject has but little relation to immunity, but this is not quite so, for adaptation is a fundamental basis of immunity, and the transmission of characters is intimately associated with the refractory state of a species.

CELLULAR METABOLISM

The metabolic process which takes place within the interior of the cell necessitates the entrance of substances of different kinds as well as the elimination of waste materials. As yet very little is known with regard to the actual mechanism of these exchanges. The only thing that is known for certain is that the electric sign of the micellae which form the cell is negative, while the surface of the cell has a positive sign. The polarization of the surface is due to the H^+ ions of acids, principally carbonic acid (H_2CO_3) which is continually being formed within the cell and which is being expelled immediately after formation. It may be deduced that the peripheral micellar layer, which plays the rôle of a membrane is permeable for cations ($+$ ions) and very weakly permeable, or impermeable, for anions ($-$ ions).

The electrical processes which take place within the cell reach their maximum at the moment of division. At this time the disposition of the micellae in characteristic radiations shows that there is a polarization of these micellae in an electric field. The centres of the radiations are negatively charged, the peripheral parts positively. The whole process is related to the intense throwing off of the carbonic acid produced at this time and which proceeds, not continuously, but in a rhythmic fashion by pulsations.

It appears that the variations in osmotic tension of the interior of cells must also play a rôle in the admission and the ejection of different substances. These variations in osmotic pressure are caused necessarily by virtue of the divisions which occur within the cell, since molecular divisions are always accompanied by an increase in osmotic pressure for a given weight of substance. Throughuot the transformation of glycogen (of which the sols possess only a very weak osmotic pressure) into glucose, then

of glucose into products still more simple, to what is the final end, water and carbonic acid, frequently occurring rhythmic variations certainly take place. The processes of the entrance and the departure of substances to or from cells must unquestionably be under the control of variations in these two factors, osmotic pressure and polarization.

Some of the unicellular organisms derive their nourishment by osmosis, others by the engulfment of prey which is digested in vacuoles. In the latter a vacuole is formed in the region occupied by the prey and a disintegration of the prey takes place. The reserve substances which it may contain—carbohydrates, fatty materials—are liberated, and later the living micellae of the body of the prey are decomposed into their constituents. The predatory protozoan acquires thus both the materials providing energy and the molecules which it incorporates into its own protoplasmic micellae, thus permitting it to manifest life and to multiply.

In the metazoa the processes of nutrition become more and more complex as the being is higher in the scale, attaining great complexity in the vertebrates. The cells of metazoa have lost the property of complete nutrition; specialization has taken place. In these complex organisms the cells are bathed in an interstitial liquid, a true internal medium, which furnishes to the cells the materials essential to their metabolism and which receives the products rejected. The exchanges between the circulating fluids, blood and lymph, and the interstitial liquid of different tissues maintain the composition of the latter fixed. The interstitial fluids, like the lymph and the blood, to the exclusion of the cells suspended in them, are moreover, entirely formed by products thrown out of the cells.

Every cell which, unlike the protozoa, lacks the power of complete nutrition, must find the food already prepared in the interstitial fluid, which, in turn, received it from the circulating fluids.

The preparation of the raw foodstuffs derived from the external world takes place in a central tube which plays the rôle of the vacuole in the protozoa. This central tube, although situated naturally within the interior of the individual, is nevertheless outside of the body properly speaking. In this tube, thanks to

the fluids secreted by the associated glands, the raw foods are broken down. The carbohydrates are liberated and broken down yielding glucose or levulose. The fats are emulsified. The protoplasmic micellae of the material are destroyed and reduced to their crystalline soluble constituents. All of these simple products pass by diffusion through the walls of the digestive tract into the circulating fluids and from there into the interstitial liquid of the different tissues where the cells are able to obtain them.

Whether such a process takes place within the vacuole of the protozoan or in the digestive tract of a mammal the mechanism is always the same; it takes place under the action of agents or principles which are termed enzymes.

ENZYMES

Payen and Persoz, in 1832, disclosed the first enzyme, amylase, in precipitating by alcohol a maceration of germinated barley. The precipitate obtained transformed by hydrolysis insoluble starch into a soluble sugar, maltose, reproducing thus the phenomenon which takes place in the grain during the course of germination. These processes are physiologically necessary, for starch can not, because of its insolubility, serve directly as food for the embryo, while maltose, being soluble, permits the embryo to start growth and to reach a stage of development sufficient to allow the young plant to remove from the air and from the soil the elements essential to the building up of its tissues.

Since that time many ferments have been discovered. Coagulating ferments have been found which destroy the colloid state and which are preliminary in their action to the enzymes of digestion. Such is casease, which can be procured from the stomach of young animals and which leads to a coagulation of the casein of the milk. However, the destruction of the colloidal state of the protein micellae of foodstuffs is not effected solely by an enzyme, but through the coöperation of the hydrochloric acid secreted by the cells of the gastric mucosa.

Other ferments, the decoagulating enzymes, rupture the material which forms the granule of the protein micella; pepsin carries the process to the peptone stage, and trypsin to the stage of the amino acids.

A fact which is of considerable importance is that the enzymes, in general, exercise an extremely specific action, that is, that they operate upon only a single substance. It is through these specialized enzymes that each of the many amino acids is degraded to a lower stage. But this is not the case with pepsin and with trypsin which act upon all protein materials indifferently.

Seventeen different enzymes have been identified in the liver of mammals up to the present time, and the number of those which still remain to be discovered is most certainly even greater. It should be noted in passing that we are forced to believe that in the liver each of the cells is able to elaborate at the same time the entire group of enzymes, or, in other words, that in this organ there are not specialized cells involved in the production of each specific enzyme.

Aside from the reservation made with reference to pepsin and trypsin (papaine is in the same class) there are enzymes which lead only to a partial destruction. For example, each of the stages in the metabolism of the nucleoproteins is effected by a specialized enzyme. The adenine and the guanine of this nucleus are decomposed by an adenase and a guanase, and the xanthine and the hypoxanthine which result are decomposed by a xanthase and an hypoxanthase. The uric acid residue is next oxidized by an urease (Schittenhelm).

Other enzymes lead to an oxidation, as is the case for laccase, to which we will return in an instant. These oxidizing enzymes are true respiratory enzymes.

Certain of the enzymes are extremely fragile, being destroyed spontaneously immediately after they leave the cell. Such is zymase of yeast, which breaks glucose down into alcohol and carbonic acid. E. Duclaux says:

Here may be seen the action of a ferment which although capable of being considered as oxidizing in that it produces carbon dioxide, is also to be regarded as a deoxidizer since it produces alcohol from the sugar. But it is indeed something more. It provides an example of the chemical rupture of a complex molecule, the sugar, into two more simple molecules; an example of that process which for a long time philosophy has reserved to the living cell, and even to a single species of living cells, namely, those of the diverse yeasts. Here the process is found for the first time separated from the domain of life, performing a chemical func-

tion appertaining to dead matter. The discovery of this ferment advances us a step further in revealing the nature of the cell. G. Bertrand has shown that the respiration of the cell, so characteristic a manifestation of life, is due to the presence of one of these secretions which is able to respire outside of the cell. E. Buchner has similarly shown that in the yeast cell the function of the alcoholic ferment, which appears to be so characteristic, belongs to a secretion of the yeast capable of breaking down the sugar quite apart from the cell from which the ferment is derived.

All of this indicates that all of those properties, termed vital, pass gradually into the realm of chemical properties capable of functioning and of being studied apart from the living intact cell. This does not abolish the cell nor life, it simply allows of their dissection and their better comprehension.

Let us note finally, and we will return to these processes in considering immunity, that the enzymes of "defense," so-called, may make their appearance when an unexpected decomposition becomes necessary. Saccharose does not normally diffuse through the walls of the digestive tract and consequently is not normally found in the blood. But if a solution of saccharose is injected into the circulation, a sucrase appears which has the ability to rupture the molecule of saccharose, with hydration, into a molecule of glucose and one of levulose.

MODE OF ACTION OF THE FERMENTS

Immediately after the discovery of the first ferments it was but natural to inquire into the composition and the mode of action of these substances. Naturally, the first attempts were by means of chemical analysis, but this method revealed nothing for it was quickly perceived that the substance isolated, and to which the name of ferment was given, was in reality a mixture of diverse substances in which the impurities were present in an amount infinitely greater than the ferment itself. Under such conditions the mass of impurities masked the true composition and the results of such analyses were very discordant. With all the ferments examination revealed, in variable quantity, carbon, oxygen, hydrogen, and nitrogen, and in addition a certain amount of various mineral substances which were considered as impurities.

In studying laccase, the oxidizing ferment found in the latex of the lac tree, G. Bertrand was the first to show that the active

principle itself was to be found precisely in those substances
which had been considered as impurities, and that it was repre-
sented by a salt of manganese. This demonstration led to a
conception in accord with which a ferment action should result
from a two-fold reaction; the ferment was formed of a system in
which two substances played a rôle, the one complementary to the
other. The active component was inorganic; the activating
component was represented as an organic substance. The in-
organic fraction, by and of itself, might be capable of effecting the
reaction, but only very slowly, while thanks to the activating
complementary substance the reaction was carried out at an
infinitely greater speed. According to Dony Henault the active
factor might well be a manganese salt, and the activating comple-
mentary substance should be, not an organic compound, but
rather a negative OH ion. This hypothesis appeared most
probable and better explained certain peculiarities. We will
shortly consider the significance of this.

Trillat, and then Dony Henault, next succeeded in preparing
an artificial ferment, producing an action analogous to that of the
natural laccase. The former incorporated traces of manganese
chloride in a slightly alkaline solution of egg albumin; the latter
by precipitating with alcohol a colloidal substance, such as dextrin
or gum dissolved in an alkaline fluid containing a manganese
salt. Thus, they provided a direct proof that the oxidizing action
of the laccase may indeed be exercised by manganese. Moreover,
even without organic support this element already possesses the
fundamental property of an oxidizing ferment. A salt of manga-
nese, or of iron, which is deoxidized by contact with organic
matter and which is reoxidized again by contact with air can be
nothing but a ferment.

All ferment activities show a number of peculiarities which
they have in common and which differentiate them sharply from
ordinary chemical reactions. Ferments act only within fairly
narrow temperature limits. With no action at all at temperatures
near 0°C. the fermentative processes become more and more
vigorous as the temperature is raised to a particular optimum
point, somewhat variable for each of the different ferments, but
situated generally at about 40°C. Above this the action is

retarded and at a fixed temperature, always below that of the boiling point of water, the activity ceases to be manifest and it can readily be proved that the ferment is then destroyed. The second characteristic is the disproportion between the quantity of the ferment which acts and the quantity of substance which is subjected to its action. Indeed, one may prepare ferment solutions containing only an infinitely small amount of dissolved material and yet they are capable of transforming in a relatively short time—a few hours—a mass of substance reaching in certain cases a million times the weight of the material, impure as it is, which we term the ferment. But it is an important fact that the decomposition is never complete. A certain proportion of the substance remains intact, and this can be shown to be due not to a weakening of the ferment in the course of its action, but to an interference, an inhibition, exercised by the accumulated products of the decomposition. In fact, if these products be removed from the medium, by whatever means, the reaction proceeds and a new portion is decomposed, so that if the products of decomposition are eliminated as fast as they are formed the action becomes complete; there no longer remains a trace of the substance against which the fermentative activity is directed. Even at this time the ferment is to be found in the medium, intact, and possessed of all its original properties. It will behave as though new.

A ferment is then an agent for the transformation of matter, capable of functioning without self-destruction, or even of attenuation, upon an unlimited quantity of material suceptible to its action. Practically, its action decreases as a result of the accumulation in the medium of decomposition products and these are inhibitory, since the ferment becomes inactive when an equilibrium between the degradation products and the transformable substance is reached. This equilibrium depends upon the absolute quantity of transformable substance, upon the quantity of the ferment, and upon the conditions, both chemical and physical, of the medium in which the reaction takes place.

REVERSIBILITY OF FERMENT ACTION

But, it may be said, all of these ferment actions of which we have seen the mechanism deal with decompositions: they are

analytical processes. What then can be the mechanism of synthesis? Our knowledge of the reversibility of ferment actions offers an explanation.

Hill was the first to show the reversibility of the fermentative process. We have already seen that ferment actions are limited, that is, that the products resulting from the transformation first retard and then, when the amount is adequate, stop ferment action. In this condition there is an equilibrium. Let us consider a concrete example, the maltase which hydrolyzes maltose by the union of a molecule of water to a molecule of maltose with the later rupture of this combination into two molecules of glucose. If we cause maltase to act in maltose solutions of differing concentrations it is found that in a 40 per cent solution the action ceases—the equilibrium is attained—when 84 per cent of the maltose has been hydrolyzed. In a 20 per cent solution the process stops when 90.5 per cent is hydrolyzed, and in a 10 per cent solution when 94.5 per cent is broken down. This is readily understood, since the greater the dilution the less marked will be the inhibiting action because the amount of glucose formed will be less. What is the cause of this state of equilibrium?

Hill has caused maltase to act, not upon maltose, but upon glucose, the final product of the maltose-maltase reaction. Working with a solution containing 40 per cent of this sugar he has observed that maltose is gradually formed from the glucose. The action is slow: after five days there is but 3.5 per cent of maltose, 10 per cent after twenty-eight days, and 15 per cent after two months.

It is now clear why the reaction is arrested. Having reached a certain state of equilibrium, varying with the conditions of the experiment, the reaction tends to change its direction. Little by little the ferment loses its aptitude for hydrolyzing maltose, and at the same time it acquires that of dehydrating glucose, thus producing maltose. The reaction carries itself in one direction or the other until the two tendencies are equal. This aptitude to reversibility appertains, moreover, as has been known for a long time, to mineral catalysers.

Since the noteworthy work of Hill (1898) the phenomenon of

reversibility has been recognized for other ferments; for lactase, for emulsine and for lipase.

The activities of living matter are accomplished generally through the intermediary of ferments. However, it is not essential, as is too often the case, to consider all of the reactions which take place within the body as fermentative in nature. We will see in the next paragraph that oxidations and reductions may occur in the absence of enzymes. In short, it appears that all decompositions are of the fermentative type, as well as the syntheses leading to the formation of crystallizable substances. With regard to synthetic actions leading to the formation of a colloid, reservations must be made, especially in that which bears upon the reversibility of action of certain non-specialized enzymes, such as papaine, trypsin, pepsin, and nuclease, all of those which, in brief, act upon the micellae or upon the protein granules.

Experiment has shown that there is a reversibility of action with certain of the strictly specialized enzymes, and there is nothing opposed to the idea that it occurs with all. But as far as the non-specialized enzymes are concerned, how can we believe that a single enzyme is able to form any of the different innumerable protein micellae which exist? Papaine, which is derived from a plant, degrades all protein micellae, animal or vegetable. Could it then form by resynthesis utilizing the amino acids, all of the proteins which are known? It is not conceivable. Further, the final synthesis of acid amines into protein micellae is effected within the cell itself. But we will see that the cells of higher organisms at least (the leucocytes excepted) contain neither pepsin nor trypsin, for these enzymes are formed only in the digestive tract itself by the union of pro-enzymes which individually lack all activity. These enzymes are unable to produce a synthesis. It is then certain that the enzymes which bring about the first act of the disintegration of proteins into amino acids can not possess a reversible action. Such a synthesis has never been demonstrated.

But, with the micellar conception of life as it has been presented above, such enzymes of final synthesis have no reason for being. The protoplasmic micella will incorporate naturally the amino acids which will form the granules of the micromicellae.

NATURE OF THE FERMENTS

We have seen above that since the first ferment was isolated chemists have attempted by analysis to reveal the secret of the particular mode of action of these forces which are always the accompaniment of life. We have also seen that the active part of a ferment may be an inorganic body. Under what particular form then, is this inorganic body to be found where it can exercise such functions and at the same time remain unchanged itself? This question now seems to be answered, for it is possible to reproduce certain fermentative actions by means of a metal or an oxide without any organic support.

We all know of the experiment of the lamp without a flame in which the alcohol is transformed by contact with the air and platinum sponge into acetic acid, or into an aldehyde, according to the degree of oxidation. Here, the platinum acts by its presence only, and not by its affinities. It awakens in the elements present latent affinities which, without it, would not manifest themselves at the temperature of the experiment. Is this not the part played by a ferment? To this action brought about by an inorganic element has been given the name of catalytic action; the active element is termed catalyzer. Fundamentally, catalyzer and ferment are two different words designating bodies exercising comparable functions; the first is the physical term, the second the biological term.

The basic factor of the activity of a catalyzer is the extent of the surface of contact. The catalytic action of a given weight of platinum, for example, will be insensible if the metal is in the form of the ingot or sheet, it becomes perceptible if the substance is reduced to foil, and it increases still further if it is in the form of sponge or especially of platinum black, that is to say, in a greater and greater state of subdivision.

We are thus returned to the question of the colloidal state. A metal in the colloidal state is capable of provoking catalytic actions and of acting as a true enzyme. Colloidal platinum, for example, decomposes, with hydration, a molecule of saccharose into two molecules, one of glucose, the other of levulose, producing in this way an effect identical to that caused by sucrase.

If it is impossible for us to penetrate the secret of the mode of action of a ferment because of the insurmountable difficulty of separating the active principle from its organic support, we have here succeeded somewhat, since here we find that the presence of a metal may suffice to carry out a fermentative function.

Experiment shows that enzymes are found in the colloidal state, an emulsion of an enzyme is a sol, and the micella of the enzyme contains both an organic particle and an inorganic particle. Ultrafiltration experiments show that this micella is very minute, and that it is relatively stable, since it resists desiccation.

As we will see, the action is exercised by the inorganic portion, but the specificity of action can not be imputed to this fraction, for we know that enzymes having very different activities have the same active complementary particle. Thus, trypsin and casease have as an inorganic portion, a calcium ion. The chlorine ion is the active complement of amylase, of protease, of maltase, and of sucrase. Furthermore, it is even possible to effect the substitution of ions without affecting the mode of action of the enzyme. In the trypsin micella it is possible to replace the calcium ion by any alkaline earth ion whatever. It follows therefore, that if the activity is brought about by the inorganic part, the specificity must result from the organic part of the micella of the enzyme.

From the rare cases where analyses have been possible upon enzymes of sufficient purity it has been demonstrated that the organic portion differs from one enzyme to another. For some of them this organic part is surely not of protein nature for it does not contain nitrogen, and it is interesting in this connection to note that these enzymes without nitrogen act upon bodies which themselves lack nitrogen, while it appears that enzymes acting upon nitrogenous bodies are likewise constituted of nitrogenous organic material. It appears, then, that there is a certain relationship between the composition of the organic portion of the enzyme and the body upon which it is operative. It is possible, and this hypothesis has been advanced, that this organic part may be of the same nature as the body decomposed, which would, up to a certain point, permit us to understand both the specificity of the action and the mode of formation.

In the micellar hypothesis of life, the granule of all enzymes

exercising a specific action should result from the dispersion of a protoplasmic micromicella.   The enzymes without strict specific action should result from a union of several dispersed micromicellae derived from different protoplasmic micellae joined together with inorganic ions.

THE ELECTRONIC REACTION

The reactions of oxidation and of reduction are those which occur most frequently in the course of life.   It is known that they may be effected through the intermediary of the enzymes, in the same way as lactase acts as an oxidation agent.   But is this the general method?   Here are some experiments which tend to show that it is not.

In 1867 Becquerel (the father of the physicist who discovered radioactivity) made the following singular observation which remained at that period inexplicable and was forgotten, without doubt because it was paradoxical and appeared to be quite contrary to the laws of chemistry.

It was known that if a solution of copper chloride ($CuCl_2$) is mixed with a solution of sodium sulfide (Na_2S) there occurs a reaction in which the only predictable products, according to the laws of chemistry, are H_2O, NaCl, and CuS.   Becquerel took a test-tube and obtained in the walls very fine capillary cracks. He placed in such a tube a solution of $CuCl_2$ and immersed this tube in a receptacle containing a solution of Na_2S.   He observed the appearance in the cupric solution of metallic Cu adherent to the walls and some cuprous chloride (Cu_2Cl_2), while in the sulfuretted solution there appeared a sulfate (Na_2SO_4) and some amorphous sulfur.

Girard and Platard have recently undertaken a study of this paradoxical reaction (using semipermeable membranes in the place of the tubes with the capillary cracks) and have arrived at the following conclusions.

Analysis of the phenomenon showed us very quickly that the determining factor of the reaction of Becquerel resided in the property of the membrane separating the two media being inequally permeable to the ions which these fluids contained.   Impermeable to the Cu^{++} ions, the membrane very readily permitted the Cl^- ions to diffuse toward the sul-

fur-containing solution, and the S^{--} ions did not pass in the reverse direction toward the chloride solution. The sulfur there, then, is in the state of amorphous sulfur. Slight variations in the pH of the copper solution occur during the course of the experiment (lasting one or two hours) and $Cu(OH)_2$ is absent.

Thus, lacking a compensatory mechanism, the selective permeability of the septum would result in an excess of anions in the sulfuretted solution, and an excess of cations in the cupric solution.

Such an electrostatic disequilibrium being impossible (the compensating mechanism which intervenes is not disclosed by any hypothesis), the appearance of neutral sulfur and of molecules of Na_2S_4 bears witness that a fraction of the S^{--} ions lose their peripheral electrons.

These electrons, abandoned by the S^{--} anions, to satisfy the menaced electrostatic equilibrium, we find again on the Cu^{++} cations which, acting then as a single electron, become fixed upon the circumference of the electronic exterior when in the state of monovalent copper as in the molecule Cu_2Cl_2, and in the state of metallic Cu when two electrons are fixed on this circumference.

The demonstration is, moreover, quantitative. From the quantity of sulfur and of sulfate formed may be deduced, and indeed one finds, corresponding quantities of Cu and of Cu_2Cl_2.

In brief, the remarkable fact is that the electrostatic disequilibrium which tends to create an elective permeability of the septum is not atoned for by a simple exchange of ions (the anions leaving the solution where the excess of negative charges appears and the cations leaving when the positive charges tend to become excessive) but by the transition of the electrons of certain anions to certain cations.

The compensating mechanism, instead of being ionic, becomes electronic.[6]

The Becquerel reaction does not take place in the chemistry of life, but Girard and Platard have effected a series of other reactions of considerable interest, for they appertain to organic chemistry and thus apply directly to the reactions of living matter.

Separate a solution of NaS_2 from a solution of fumaric acid by a semipermeable membrane. At a temperature of $15°$ at the end of twenty hours a sulfate appears in the sulfuretted solution and in the fumaric solution will be found some sulfur, a little H_2S, and some succinic acid. Here there is accomplished the appearance of a new organic molecule without the intervention of a chemical or fermentative reducing agent. The reaction is due to the unequally rapid passage of different ions through the

[6] Compt. rend. Soc. de biol., 1924, **90**, 932.

membrane. The Na^+ cations pass easily and quickly, the H^+ cations more slowly, and the S^{--} anions very slowly.

Another reaction may be mentioned. A parchment membrane separates a solution of iron sulfate $(Fe_2(SO_4)_3)$ from an alkaline solution of sodium fumarate. At the end of about 20 hours a reduction may be observed in the ferric solution. As for the initial solution of fumarate where no trace of iron has diffused, one can demonstrate the presence of tartaric acid. If the experiment is prolonged oxidation processes take place, up to the point of the appearance of formate and of carbonate.

In this last reaction, the process is due to the fact that the passage of SO_4^{--} anions is favored with respect to that of the OH^- anions. There is an electrostatic disequilibrium. In the solution where the excess of anions tends to appear, the OH^- ions in becoming fixed on either side of the ethylenic double bond of the organic molecule lose electrons which are gathered in by the ferric ions.

It may be noted that this mechanism of oxidation-reduction, to which the analogy with biochemical processes of the same nature is apparent, does not require any condition other than that the wall separating the solutions concerned have the property of elective permeability for the ions of the two media. But, precisely this property is possessed by the walls of living cells to a remarkable degree. The demonstrations given by Mestrezat, and by V. Morax and by one of us, leave no room for doubt in this respect.[7]

These experiments show, that in addition to the fermentative reactions other reactions involving electronic equilibrium must enter into the chemistry of living matter. The difference between these two modes of action is possibly more apparent than real.

As these authors whom I have cited have remarked, the principal factor of the reaction is the physical state of the membrane, and, in so far as this deals with cellular reactions, one other remark is requisite. The permeability of a cellular wall is not stable, but varies continually. And since the reactions of electronic disequilibrium are determined solely by permeability, the reactions will not always be the same for a single cell, or for the same reacting bodies. The result then will be variable according

[7] Girard and Platard, loc. cit.

to the cellular conditions of the moment. The reaction is in reality determined by the colloidal state of the cellular wall. Here is an enormous field for investigation.

CONCLUSIONS

We are now, at the end of this long discussion, arrived at the point where we can see that it is impossible to speak of any action taking place within a living being without taking account of the colloidal state which we see intervenes in every one of the intimate phenomena of life.

Life is the result of a particular constitution of a colloidal micella, the protoplasmic micella, and this resultant consists in a union of two properties, the powers of assimilation and of adaptation, which implies, the one, the possibility of multiplication, the other, the possibility of continual variability.

All of the reactions which take place within a living being owe their characteristics to the colloidal state. Each micella, even though inorganic, undergoes automatically an adaptation to its medium, for it can only subsist when in an equilibrium with the conditions of this medium. As long as the conditions of the medium are compatible with the colloidal state the micella adapts itself. When the variations of the medium become too profound, when the conditions cease to be compatible with the colloidal state, coagulation results and the micellae "die." When the micellae of a living being flocculate because of the conditions of the medium, the being dies, for only the colloidal state is compatible with life.

Study of the reactions of living matter under the influence of certain conditions special to the internal medium, resulting from the presence within the organism of a living being foreign to this organism, constitutes the science termed immunology. All of these reactions can necessarily be reduced to colloidal reactions, which may be divided into two categories.

The primary specific reactions which are accomplished through the instrumentality of specific enzymes are reactions which tend to maintain the colloidal equilibrium, that is, the conservation of life. These are the specific reactions of prophylaxis—of immunity.

But these specific reactions lead to secondary phenomena which

affect the micellar stability in the direction of a flocculation or of a dissemination. These reactions are non-specific and tend to destroy the colloidal state, and, as a result, tend toward the disorganization of living matter. Thus they tend toward death, and the organism within which they take place is "diseased." These are the reactions of contra-immunity,—of anaphylaxis.

According to the dominance of the first or the second type of reaction the organism survives or succumbs.

CHAPTER II

The Possibility of the Specific Reaction

RESERVES OF ENERGY

Living beings are able to subsist only on condition that they exercise a constant defense against the destructive influences of their environment. This defense is active, and therefore requires the expenditure of energy, which of necessity must be derived from the environment. The basis of defense is, therefore, the power of assimilation.

But the living being, the animal in particular, is likely to find itself at any moment without food because of its absence from his reach. He must, therefore, maintain himself for a certain length of time during which food may be discovered. It is essential, then, that it possess the faculty of accumulating reserves which will furnish the energy which must be expended in the continual struggle against the environment. Such reserves are present even in the most rudimentary organisms. The yeasts store glycogen, and the microscope shows that many bacteria have inclusions of fatty materials within their protoplasm. It appears, then, to be a general law.

Not only the solid or liquid foodstuffs are capable of being placed in reserve within the tissues of the living organism, but certain forms are able to accumulate reserves of oxygen. The importance of this fact was first brought out by Verworn. Anna Drzewina has shown that certain invertebrates—actinia, worms, caterpillars—and even the embryos of vertebrates, of the frog for example, can live for several days in a tube free of oxygen. When they have consumed all of the oxygen held in reserve in their tissues they fall into a state of narcosis, of anesthesia, and of paralysis. These manifestations are explained by the requirements of the nerve cells, which are greater as regards oxygen than are the cells of the other tissues. When an adult frog is placed at a temperature of 32° to 35°C. it falls into a state of paralysis after having presented

a series of muscular contractions. Winterstein has shown that
these symptoms are produced by asphyxia of the nervous system
caused by the impoverishment of the oxygen of the tissues which,
at too high a temperature, consume the gas too rapidly.

Certain plants of desert regions can accumulate reserves of
water. Such is the case with the agave and the cactus which hold
very large quantities of water in the spongy tissues of the leaves.
In animals exposed to deprivation of water, the defense consists
rather in reducing to a minimum the excretion of fluid.

More interesting is the situation with regard to reserves of carbo-
hydrates and fats, for this exemplifies a general law.

The expenditure of energy which all living beings are called
upon to effect is incessant, but it is not uniform. It varies accord-
ing to the destructive forces of the environment; weak at certain
periods it increases enormously at times when the organism must
accomplish an effort within a few instants. If it weakens at this
precise moment, if the source of energy immediately available is
exhausted, death results. All living organisms must then hold in
reserve a certain quantity of matter ready for use at the moment of
need in order to make at any time the necessary defensive effort.
And this necessity for reserves is the greater the more fragile the
being, and fragility is synonomous with complexity.

Among plants the reserves serve principally to assure the conser-
vation of the species; either they must be accumulated in differ-
ent parts of the plant to enable it to meet the demand for energy
requisite for fructescence, or they must be placed directly within
reach of the embryo in the seeds and in the fruit.

Vegetarian animals absorb these reserves, which thus furnish
them with all the necessary substances; reserves of energy, repre-
sented by the carbohydrates and fats, and the substances neces-
sary to the building up of their cells, represented by the amino
acids which are present in the nitrogenous substances. The
vegetarian, and the same is true for the carnivore which is parasitic
in the second degree, must accumulate these reserves in two forms,
as glycogen and as fats.

Glycogen constitutes the reserve element which can be mobi-
lized immediately, and is in the greater part stored in the liver
(11 per cent of the weight of the organ) and in the muscles (0.5

to 1 per cent). This glycogen originates in the carbohydrates and the albuminous substances; it is a colloidal carbohydrate which, as such, can not be utilized directly by the cell. It is transformed into glucose at the moment of its utilization, and the glucose is carried by the blood to the different tissues.

The fats constitute the great fund of reserve energy. Contrary to the carbohydrates which are always accumulated in the form of glycogen, the fats may be stored in the tissues without profound modification, as is shown by causing two dogs previously deprived of their fatty reserves by fasting, to ingest, the first, lean meat and linseed oil, and the second, lean meat and sheep fat. The two dogs thrive and become fattened and it can be shown that the reserves of the first are composed of an oil which remains fluid at 0°C., as does linseed oil, while those of the second consist of a fat which, like mutton tallow, is still a solid at a temperature of 50°C. This peculiarity is applicable, however, only to the reserve fat, for it can be readily shown that the fat which enters into the constitution, whatever may have been its origin, is always "dog fat." This last is a synthetic fat and it is this alone which can be utilized. The reserve fats are modified before their use.

The fatty reserves may be constituted, not only from the fats ingested but they may be built up through a series of decompositions and reconstructions by the ferments of the organism from carbohydrates and nitrogenous substances. The organism in its haste to replenish its reserves, stores, then, native fats, without taking the time to give them the final structure in which they can be utilized.

Can we see in all of these phenomena a "foresight" on the part of the organism in preparation for a possible struggle? No indeed, and this is shown very well by the fact that organisms often amass, even at the expense of the use of a considerable amount of energy, reserves which are of no value, or indeed, reserves which may be harmful to them.

Fruits contain a very important amount of carbohydrate, of tannin, and of fats, which are of no utility for the seeds, for the pulp of the fruit has for a long time been decomposed and has disappeared when the seeds start to germinate. The fleshy pericarp is therefore useless to the plant. Bohn has noted that

the considerable chemical activity displayed by the plant correlatively with the formation of the seed appears to be the result of the reaction of the flower in opposition to the excitation of the embryo, to irritation caused by the latter, and he offers some proof of this. One can observe in nature the formation of reserves of which the determining cause is not the egg of the plant but the egg of an animal. The result is then a tumor, a gall, instead of a fruit to which it presents certain resemblances. Certain Diptera, the Cecidomyia, depositing their eggs in plants belonging to different genera, Hypericum, Galium, Vicia, lead to the formation of false fruits presenting the form of pods or diachenia. The resemblance is not limited to the external form, since the pseudo-fruit has the chemical composition and the histological structure of a true fruit. Russell has shown that in the galls of the Cypnips tannin, starch, fats, and even sugar are found in contact with the larva in the so-called alimentary mass. Here are, then, plants which accumulate food reserves, not only useless but actually harmful to themselves, since they provide a food supply for the parasitic larvae.

Nothing exists in nature in view of a predetermined end. All reaction is only the inevitable consequence of a series of phenomena of a physical order. If this reaction is useful to the being in which the circumstances of the moment cause it to be produced the being is viable and may be the origin of a line of like beings, for the same favorable circumstances will necessarily reproduce themselves in the descendants, at least while the conditions of the environment remain the same or vary only within the limits compatible with the reaction under consideration. If, on the contrary, the conditions of the moment are such that the reaction can not take place the being disappears.

The harmony of nature is only a poetic expression, wide of the mark and belied by the facts. It appeals to us only because we have not witnessed the formation and the almost simultaneous disappearance of an infinite number of developmental undertakings which were not viable. A being, the first of its line, as a result of favorable environmental circumstances at the moment of its formation, may possibly accumulate reserves. That we comprehend, and we speak of a defense. The thing which we are not

able to realize is that billions of beings have disappeared, simply because at the moment of their formation the conditions of their environment were not contributory to the acquisition of the faculty of accumulating reserves.   These billions of beings were unarmed, they were deprived of the possibility of immediate and constant defense, hence they succumbed.

It is not exaggeration to maintain that the actual existing species constitute only an infinitesimal fraction of those which have been born, and probably are born each day, and which escape our attention for they are unable to live.   Only a small number of forms are viable; those which do not present too great discords with the mechanisms which assure their conservation.   These disharmonies and the defective misfits are the rule in nature.   Harmonious systems are the exception (Loeb).

CHAPTER III

The Consequences of the Specific Reaction

THE CONFLICT BETWEEN SPECIES

It is easily possible to imagine the existence of an inorganic body independent of any environmental medium whatever, but for the living being such a conception is impossible. The idea of life is inseparable from the idea of an environment which provides in some fashion for the maintenance of that life. It is from this environment that the being incessantly procures the elements which permit it to remain alive. This participation of the environment is even the origin of life. We have seen that the micellae owe their special properties to the absorbed ions which give an electrical charge to the surface of the particle. These ions are located in a layer of surrounding fluid which adheres to the particle because of the phenomenon of surface tension. This layer of liquid ceases, then, to be a part of the environment, for it is incorporated into the particle. It is the same with the ions which are likewise furnished by the environment; this union from which the micella results may be considered as the beginning and the origin of the active struggle against the environment.

Since the time of Lamarck many theories have been brought forth to explain evolution, but analysis of these diverse theories immediately shows that, despite all care taken by their authors to avoid it, they are all dominated by the conception of Lamarck—adaptation to the environment.[1] Natural selection, mutation, are only corollaries to the principle of adaptation. Lamarck certainly saw correctly when he attributed to adaptation to the environment a preponderant rôle in the processes of evolution; at the basis of the fundamental cause of the survival of a line, of a species, rests the aptitude for its defense. Fundamentally,

[1] Without forgetting the continuers of the work of Lamarck, Geoffrey Saint-Hilaire, and the leaders of the American school, Hyatt, Packard, and Cope.

adaptation and survival are the consequences of defense, or, to speak more correctly, of a victorious "specific reaction."

The elimination of certain species, which occurred so frequently in geologic time, has always been due either to an impossibility of defense against a too sudden change in the conditions of the environment, for example, a fall or an elevation in temperature, or prolonged drought, which did not give the being sufficient time to adapt itself to the new conditions, or to a breaking down of the defense through the competition or the parasitism exercised by other species.

The defense against competition, when the conditions of the environment are equally favorable to both species concerned, is in general a very simple mechanism which needs no explanation; brute force determines the outcome. On the contrary, when the environmental conditions favor but one of the species, that form quickly predominates.

Parasitism is of prime importance, since in all epochs it has formed the principal factor in the elimination of isolated individuals and of species. It occurs among the predatory creatures, individuals of the same order of magnitude, and among the infinitely small, the bacteria.

It is needless to emphasize the fact of the elimination of the individual; contagious diseases—human, animal, and vegetable—transform each day billions of living beings into inanimate matter.

The rôle of contagious disease in the complete extinction of species through geologic time has certainly been dominant; it is, moreover, still operative today, and will be active necessarily in the future. Here is an example which dates back but a few years. During the first half of the last century the passenger pigeon was extremely common in North America. It is sufficient to state in order to give an idea of its abundance, that in Canada and the adjoining regions of the United States, each village possessed small howitzers, which, at the period of the passage of the migratory birds, were filled with small stones as projectiles and fired, without aiming, toward the sky. The victims were not consumed, there were too many, but they served to prepare the stores of fat for the rest of the year. But, within the space of one year the species were annihilated, without a single survivor. Some

years later the Government of the United States offered a very
material reward for the capture of a single couple of passenger
pigeons, but it was impossible to find a single one.

In certain cases it is possible to utilize bacterial parasitism as a
means for fighting animals which are harmful. In this, as in all
that concerns the domain of microbiology, the way was disclosed
by Pasteur, who showed that it was possible to start fatal epizo-
otics among the rabbits which abounded in certain sections of the
country by utilizing the bacterium of chicken cholera. Inspired
by this work, d'Herelle has brought about epizootics among the
swarms of locusts which ravage a number of the warm regions of
the world by infecting them with cultures of a bacillus, *Bacillus
acridiorum*, which had been isolated during a spontaneous epizo-
otic of locusts studied in Mexico.

EVOLUTION AND SYMBIOSIS

Evolution results not only from an adaptation to adverse con-
ditions in the environment, but is also consequent upon a com-
promise which terminates in a symbiosis of two competing species.
We owe this last conception to Noël Bernard, in support of
which he has furnished a number of the most convincing proofs.
The evolutionary theory of Noël Bernard supplements that of
Lamarck.[2]

Symbiosis is a phenomenon universally distributed throughout
the vegetable kingdom. The whole group of the lichens results
from the union of an alga and a fungus. The majority of the
Hepatica have a thallus regularly invaded by filamentous fungi.
Indeed, a large number of vascular plants normally harbor sym-
biotic fungi in their roots.

One might wish to see in these associations a mutual harmony
between the two beings united to the end of a common benefit.
According to Noël Bernard the reciprocal actions of the two beings
concerned do not differ from those which are operative between
a superior animal and a pathogenic bacterium which attempts to
invade it. He has shown, moreover, "that symbiosis intervenes

[2] With reference to this subject, see, aside from the papers of Noël
Bernard, the excellent review of J. Magrou, published in the Bulletin de
l'Institut Pasteur, 1922, **20**, 169.

to modify the development of plants which find themselves subjected to it, and it is thus able to play an essential rôle in the formation and in the evolution of vegetable species."

Among other things, Noël Bernard has shown that the germination of the seeds of the Orchidaceae can not be effected, in many species, unless they have been previously invaded by fungi. From this parasitism one of three results may ensue; the embryo may offer no defense to the invasion, whereupon it succumbs, or, after the beginning of the invasion the embryo may destroy the microorganism, thanks to a process of cellular digestion analogous to phagocytosis, or, finally, there may result a symbiosis between the embryo and the fungus. Here, phagocytosis is still operative, but only partially, and an equilibrium is established between the defense and the attack, and in this case only is the embryo viable. This diversity of result depends upon the degree of virulence of the fungus, a virulence subject to increase by passage through the seeds of the Orchidaceae. It is clear from these demonstrations that symbiosis is, indeed, to adopt the expression of Noël Bernard, on the "frontier of disease." Germination of the Orchidaceae seed depends, then, upon the issue of this struggle, the means of defense and of attack of the two organisms involved constituting the opposing forces.

Comparable facts have been established for other plants, the plants with tubercles among others, and from the sum of the investigations it appears that in symbiosis the parasite maintains itself through its virulence, which, in the last analysis, is shown by the secretion of digestive ferments which allow it to utilize the tissues of the host for its development; the plant, on its side, defends itself and limits the development of the parasite through phagocytosis and by the production of antiferments.

But if symbiosis is only a particular case of infection, it ought to manifest itself by symptoms, and this symbiosis being normal in certain species the symptoms ought to constitute the characteristics of these species. Noël Bernard has shown that one of these symptoms is tubercularization, that is, the formation of tubercles.

Aside from the specific characters which belong by nature to the germ of a species, there exist others which depend upon external factors, and if one could vary these one ought to be able to modify

or degenerate the corresponding characters. One may modify experimentally the development of certain species of the Orchidaceae (Noël Bernard), or form new species of lichens (Moreau), by changing the normal symbiotic conditions. The abrupt mutations of De Vries are the result of adaptation to parasitism.

From the sum of these experiments Noël Bernard has been induced to affirm:

Annual plants attacked, at first accidentally, by fungi have ceased to flower in their first year and by compensation, the lateral shoots of their stalk have given birth to perennial organs, bulbs, or branches of rhizome. The formation of these organs should next become more and more precocious, at the same time that the association with the fungi becomes during each year more prolonged and more intimate. The development of vascular plants must have been the consequence of a high degree of adaptation of certain Muscineae to a life of symbiosis with the fungi.

These hypotheses, based however upon facts, have since been substantiated by the experiments of many authors.

Symbiosis may, in addition, act as an evolutionary factor among the Bacteriaceae. Pinoy has shown that with Myxobacterium sporulation varies in accordance with the associated bacterial species, and it appears from the experiments of d'Herelle that bacterial mutations may be effected through parasitism by an ultramicrobe, the bacteriophage.

Not only in the vegetable kingdom has symbiosis been observed, for numerous cases of symbiosis have already been noted between an animal and a bacterium, and new ones are discovered each year. Cells with bacteria have been noted among the insects in the Orthoptera and the Diptera (Roubaud), the Mycetoma of Coleoptera and of Hemiptera, and of Pediculus. Among the phosphorescent animals the luminous property of certain organs is due to a "luminous symbiosis" with the phosphorescent bacteria. It has been known for a long time that luminous bacteria are present in sea water, from which they have been isolated and cultivated. It has recently been discovered that in certain fish (N. Harvey) and in cephalopod molluscs (Pierrantoni) the luminous organs are formed of glandular tubules packed with these bacteria. It is certain that in these symbiotic relationships the associated bacterium

exercises an effect upon the organism of the host and has an influence upon its evolution.

In man himself, for example, it is recognized that the spirochete of syphilis is the cause of certain hereditary characters. It may only require that the symbiosis become habitual, such as that seen among botanic and zoologic species, for the human race to undergo an evolution, by sudden mutations, which may profoundly modify its characters.

Symbiosis has still another significance when we consider that the defense of the susceptible organism against the bacteria is assured by an ultramicrobe, parasitic on the bacteria, which is a normal inhabitant of the body throughout the animal series. This is still a symbiosis, although it differs from those which we have noted above. Here, the ultramicrobe lives in symbiosis with the normal bacterial flora of the intestinal tract (giving to the word symbiosis the meaning attributed to it by Noël Bernard) but it is capable of penetrating within the tissues so that it may there exercise its protective action.

Let us note further, that in a symbiosis it is not only the characteristics of the host which are modified; those of the parasite which adapt itself to the common life are equally modified. Our attention is especially attracted to the first because they are the most evident simply because of the respective size of the two beings involved.

Fundamentally, the possibility of defense decides, not only the fate of the species of vegetable or animal, but it also determines the sum total of its characteristics.

In brief, the phenomenon of adaptation to the environment, which explains all of the processes of evolution, presents itself from a twofold point of view. On the one side, under the influence of physical causes, resulting from variations in the external environment (Lamarck) and on the other side, under the influence of chemical causes, resulting from variations in the internal medium provoked by symbiosis (Noël Bernard) the being acquires new characters which permit it to resist new conditions in which it is called upon to live. This is, however, only a statement of words. May we go further yet and attempt to catch a glimpse of the mechanism of adaptation?

MICELLAR SPECIFICITY

From the point of view of any of the hypotheses concerning the nature of living matter, whether it be the cellular or the micellar theory, one fact is absolutely certain and universally accepted, namely that the cell is constituted of an assemblage of micellae. Hence the first act in the processes of evolution must necessarily be a modification of the fundamental micella, this transformation reflecting itself in the functioning of the cell and consequently in the entire organism.

We will see in the course of this discussion how inexorable is the struggle of the organism against colloids of foreign origin, the intrusion of which tends to obscure its individuality. These reactions are sometimes so violent that the struggle for integrity may involve the death of the individual. It is not strange, then, that under the influence of a symbiosis, in which the colloids always play a part, the characteristics of an organism may be modified profoundly. Adaptation is a process of defense. Victory for the individual means survival, but it is obtained only at the expense of its integrity.

The specificity of each living being is an absolute principle;[3] each particle of an organism bears the imprint of the individual from which it originated. This strict specificity is not only a specificity of matter, but is also a specificity of physical state, and although this specificity is incomprehensible if one considers the unchanging chemical molecule as the fundamental unit of living matter, it becomes natural and indeed inevitable when one recognizes that the fundamental unit is the colloidal micella.

A molecule of an amino acid is always replaceable by another molecule of the same amino acid, as is shown by the decomposition of any vegetable or animal albumin whatsoever. They are all interchangeable and the cell assimilates them without distinction as to their origin. On the contrary, one micella may differ from another micella by the chemical constitution of the molecules of which it is composed and by its charge of electricity which varies

[3] We will see that this specificity is experimentally demonstrable. A tissue of a given animal belonging to a given species is not of necessity identical with the same tissue of another animal of the same species.

according to the chemical constitution and the number of the ions which enter into its composition. A micella formed of molecules of amino acids and bearing its electrical charge in the form of ions, may then fail of being identical with any other micella, the nucleus of which is, nevertheless, formed of the same amino acids. The micella may, indeed, differ at two different moments, for the equilibrium of its constituents varies continually as a function of the environment.

Specificity becomes, then, easy to understand, even in the case of two animals of the same species, for one micella of a cell of a sheep, for example, although it presents the same chemical constitution as a micella of a cell symmetrically placed in another sheep, is able to differ appreciably in accordance with its electrical charge, and by virtue of the ions which cause this charge, since the nature and number of the latter varies according to the composition of the intermicellar liquid, and since infinitesimal modifications in the composition of this liquid suffice to provoke differences in charge. But this composition varies continually, even in a given animal, hence the greater the reason that it is different in another individual.

Let us mention in passing that in consideration of all of the analyses which have been made upon vegetable and animal tissues belonging to individuals of different ages, it appears that old age is associated with a substitution of calcium ions for those of potassium. In fact, it may be observed, both in vegetable and in animal matter, that the proportion of potassium diminishes progressively throughout the course of life, while there is a corresponding increase in the proportion of calcium. But, we know that the calcium ion always exercises a coagulating action.

Various authors, Lumière among others, have attributed old age to the natural tendency of all colloids toward coagulation. In reality, the progressive tendency toward a coagulation of the protoplasmic micellae is indeed most assuredly the cause of old age, but this tendency results from the progressive substitution of Ca^{++} ions for those of K^+ ions. It may not be said that a day will never come when this substitution can be inhibited, or even when the reverse substitution can be accomplished in the aged.

In this respect the following remark may be of interest. It is

known that the radioactivity of certain elements results from the disintegration of an atom which changes spontaneously and with great violence and projects into space one of the fragments of its nucleus. These fragments are either electrons (β rays) or atoms of helium bearing two quanta of positive electricity (α rays). This fragmentation liberates considerable energy. In losing one of its particles the atom is transformed into another; a transmutation occurs. It is thus that, as a result of a series of successive transmutations, necessitating, however, several thousands of years for its completion, uranium is changed into lead. Radium is one of the stages of the change.

The potassium ion is constant in all tissues, and Zwaardemaker has advanced the hypothesis that activity may depend upon the radioactivity of potassium, the single radioactive element existing in the body. But, here is a fact which is curious and which, I think has not yet been noted, the radioactive potassium element being particularly abundant in the young cell, should, according to the laws of transmutation, be transformed into calcium, the element of old cells, non-radioactive, and liberating no energy. This may be more than a coincidence. Obviously I do not think that the calcium of old cells results from the transmutation of the potassium of the young cells, since in nature such an integral transmutation would require thousands of centuries, but it is nevertheless true that continually, every second, there is produced in us a transmutation of a certain number of atoms of potassium into calcium with the liberation of energy, and such a phenomenon might be able to direct a slow substitution of ions.

ADAPTATION OF MICELLAE

May we discover why the micellae which compose a cell are harmonious? May we ascertain why it is that a cell is susceptible of adaptation?

The micella forms within the cell itself, and its equilibrium depends upon the conditions of the intermicellar liquid at the moment of its formation. All new micellae formed will necessarily be comparable to the neighboring micellae to be found under identical conditions as to the medium. But if the conditions of the liquid vary in a progressive fashion during the period of

formation the constitution of the micellae of this single cell will vary of necessity in parallel with the ions which form them. For each micella there is not a point of equilibrium, but a zone of equilibrium compatible with its existence as a micella. This is shown by direct experimentation with colloidal solutions, as we have seen elsewhere.

Into a solution of an alkaline citrate of an optimum concentration let us introduce precipitated barium sulfate. Under the influence of the negative citric ions a fragmentation of the particles of the precipitate of barium sulfate takes place with the formation of micellae through the adsorption of negative ions by each of these fragments. Let us add to the liquid a new quantity of citrate. In accordance with the new concentration a change of equilibrium in the micellae will occur, in the direction of a flocculation, and this by virtue of the replacement of positive metallic ions with an equal number of negative citric ions. But if the latter concentration differs only but little from the original the existence of the micellae will not be compromised; only the electric charge borne by each of them will vary. These are no longer, however, the same micellae as those present before the increase in the amount of citrate. Their chemical composition will be different as the result of the adsorption of variable amounts of negative citric ions or of metallic positive ions. Their physical properties will no longer be the same because of the change in the electric charge resulting from the adsorption of ions. There exists, then, for each micella, not a point of equilibrium but a zone of equilibrium compatible with the existence of the micella. All of the properties of a cell, including its functions, depend necessarily upon the equilibrium conditions of its constituent micellae. That is to say, the ions adsorbed by the micellae may change in number and in nature, and all change leads to a variation in polarization in these micellae, and, consequently, in the polarization of the entire cell. Every time that the medium changes in any manner the micellae adapt themselves, or if adaptation is impossible they are destroyed, they die.

But this explains only the minor variations, those which are reversible. Considering it solely from the point of view of the physico-chemical reactions of the colloidal state, a more profound

modification of the protoplasmic micella can be comprehended, and even irreversible modifications, affecting the internal constitution of the micella without necessarily being attended by death.

Freundlich has shown that if a given quantity of a negative colloid is precipitated by the addition of a determined quantity of a positive colloid when added *all at once*, such a flocculation does not take place if the addition is made very slowly, a drop at a time throughout a period of a few hours. In order to cause flocculation of the negative colloid by this procedure it is necessary to add two, or even three times that amount of material which would have caused flocculation if added suddenly.

And this is precisely the case in symbiosis, where two different colloidal beings, each the sources of differing colloids—in a word, two colloidal systems—react one on the other. Freundlich, without apparently having suspected it, has accomplished *in vitro* the process of evolution.

Let us suppose the formation of successive micellae within a fluid whose composition is changing slowly under the influence of environmental conditions. It is the same thing as in the case of the micellae formed within a cell at different moments. There may be four types of micellae formed at these different moments, B, C, D, and E. Let us designate the conditions, in their entirety, of the intermicellar liquid at the moment of their formation respectively by a', a, b, c, d, e, f, and g; b representing the conditions of the medium at the moment of the formation of B, c, at the moment of the formation of C, and so on. Let us suppose that the zone of equilibrium of B extends from a' to d. The conditions of the medium, although they may vary, will still permit the existence of this micella B up to the time of the formation of C, for which the zone of formation will be from a to e. B and C coexist up to the moment of the formation of D, whose zone will be from b to f. At this moment B ceases to exist, but the life of the cell is continued nevertheless, for micellae of the D type replace them. After a certain period micellae of the E type are formed; their zone of equilibrium will be from c to g, and they will replace the micellae of the C type. Thus it is clear that the cell will continue to exist despite the successive transformations, for only at the moment e will micellae be formed different from those which com-

posed it at the moment *b* and whose functions must be different. Thus it will adapt itself.

The maximum of variation is necessarily produced as a result of a symbiosis. In any given organism the internal medium, with which the micellae are in continual equilibrium, will not be the medium of a single other being. But the two beings in symbiosis obtain there the materials which are necessary to them, and there both discharge the products of their metabolism, the substances required and the substances poured out differing for each of the two. The nature of the internal medium will, therefore, change profoundly, and if adaptation of the protoplasmic micellae of the two beings to the new conditions of the medium is possible, each of the constituent micellae of the two beings will come into equilibrium with this new medium, varying in accord with its constitution.

The functions of the cell are only the resultant of the state of the constituent micellae, and the organism as a whole, morphologically and physiologically, is simply the resultant of the sum of the functions of the cells. The complete being, or rather, each of the two beings in symbiosis will be changed the more deeply as the micellar adaptation has been the more profound.

The place which has been accorded to symbiosis in the general scheme of living beings may appear strange to those unfamiliar with the biological research of the last thirty years. This has shown that symbiosis does not form the exception in nature, but that it is a rule which appears to be more and more general. We will have occasion to cite examples of it in the course of this discussion.

It might likewise be considered strange that, in a work professing to be a study of immunity, such questions should be subjected to examination, questions which, at first sight, appear to be entirely foreign to the subject. But this is not so. All of the processes of symbiotic adaptation are strictly within the domain of immunology. For whenever the adaptation is not perfect between the two beings involved, just so far is a final equilibrium not established, and the symbiosis is disease. The primordial act of evolution is a phenomenon of immunity.

The possibility of adaptation possessed by all living beings, from

which the virulence of the bacterium is derived; the possibility of resistance in the parasitized being, the acquisition of the refractory state, the adaptation to the physical conditions of the external medium, all of these properties are owing to the colloidal state. These properties permit us to understand that adaptation of the cell may be transitory or permanent; that the cell may vary throughout its life both from the chemical and physiological points of view as a result of the union of the organic micellae with different metallic ions. It is possible, finally, to understand the mechanism of living beings because of the variations in the conditions of their environment, the transmission of acquired characteristics, and even the sudden mutations which take place under the influence of symbiosis.

However this may be, the micellae of one individual are not identical absolutely with the corresponding micellae of another individual of the same species, for these two individuals have not been subjected to the same conditions. Each of them has had to struggle against the colloids secreted by different species of pathogenic bacteria; each of them has acquired different immunities, thanks to adaptation, and this adaptation has left its imprint upon the constitution of those groups of cells which have been burdened with the efforts of the struggle.

With individuals belonging to different, but related, species these differences will be perforce accentuated, for it is certain that evolution which has led to the separation of species, has been simply a result of an evolution of the micellae constituting a group of cells in response to the different conditions of the environment undergone by the ancestors, the first of each of the lines. The more distant the species are from each other, the more dissimilar will be the micellae constituting the cells of the individuals which compose them. The maximum of heterogeneity is to be observed between the micellae of individuals placed at the two extremes of the scale of being—the bacterium, or rather the ultramicrobe, and man.

PART TWO

THE REACTION AGAINST INANIMATE AGENTS

CHAPTER I

The Reaction Against Crystalloids

INANIMATE AGENTS

It hardly appears essential to consider in this work, especially devoted to the study of immunity, the mode of reaction of living beings to variations in the conditions in which they live, such as variations in temperature, in moisture, and in pressure. On the contrary, to permit a general understanding of the mode of reaction, it is desirable to note the way in which organisms react to changes brought about in the internal medium, that is to say, in the liquid which bathes the cells, by the action of crystalloids such as may accidentally penetrate there and which may modify its composition. This brief statement serves as an introduction to the study, much more complex of the modifications of this internal medium resulting from the penetration into the organism of substances in the colloidal state.

The classification of different substances into foods and poisons is indeed impossible, for the differences are quantitative rather than qualitative. The difficulty is increased by the fact that we do not know but what an element, considered as toxic in a large dose, may not be an indispensable food in a small dose, as is the case, for example, with arsenic. The question becomes still further complicated with albuminous substances, the foods par excellence, but which when directly introduced into the interior of the body, even in infinitesimal dosage, may act as poisons and induce very peculiar reactionary processes. In view of the considerable importance of the phenomena related to the conflict which arises between living beings and these albuminous substances, which are all of colloid nature, let us examine in detail the reactions which they induce.[1]

In the case of the albuminous substance, even if the proteins

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[1] In Chapter I we have noted the differences between crystalloids and colloids.

involved are non-living, a study of the reactions brought about by their presence is in the realm of immunology, for it must not be forgotten that a bacterium is made up of matter wholly in the colloidal state, and that the products of its metabolism thrown out into the internal medium of the being which it parasitizes are also in large part colloidal.

A bacterium acts, not solely because it is living, but also because it is a colloid and the source of colloids. Before proceeding to consider the most complicated case, that of the reactions provoked by the presence of one living being within another, it will be well to consider the phenomena which result from the presence of proteins which, even if they are not living, are still in the colloidal state, that is, in the same physical state as the substance of living beings.

FOODS AND POISONS

It is often very difficult to determine what is, for a given organism, the entire number of indispensable elements which it must procure from its environment in order to maintain life. This difficulty increases as the being becomes higher in the scale of organization. Investigation in this direction has been very active, particularly in American laboratories, under the stimulus provided by the discovery of those still mysterious substances, if indeed they be substances, which have been termed "vitamines," and which are perhaps only physical properties. However that may be, the complete study of the food requirements of a living being has been accomplished for one of the most common molds, *Aspergillus niger*, which develops spontaneously upon all organic acid materials, on portions of cut fruit for example. This investigation by Raulin, carried out very methodically and with remarkable precision, is now about fifty years old. This scientist was able to prepare a medium, ideal so to speak, permitting the maximum growth of this mold. By suppressing certain elements in the medium he showed the extreme importance of infinitely small amounts of certain metallic salts, which in higher concentration, were toxic. The suppression of zinc in the medium, for example, although it should be present in an amount of only 20 mgm. per liter, reduced the yield of the Aspergillus by nine times.

"Upon the use of such infinitesimal proportions of an element," observes Duclaux, "does the health of a living being depend!" What is the rôle of these minute quantities of metallic salts? Although the direct proof has not yet been furnished, what we know of ferment action allows us to suppose that they must act as catalyzers in the constitution of the cellular ferments.

The vegetable is more sensitive to the action of harmful elements present in a medium than it is to a deprivation of useful elements. Germination of the spores of *Aspergillus niger*, for example, is impossible if one adds to the nutritional fluid one part in sixteen hundred thousand of a silver salt. Germination will not begin in a silver vessel, although chemical analysis can not demonstrate that any of the vessel is dissolved in the fluid.

The line of demarcation between the antiseptic and the food is, then, extremely uncertain. Corrosive sublimate is the antiseptic par excellence, but weak traces of sublimate instead of preventing the growth of certain bacteria definitely favor it indicating that the bacterium utilizes this salt. If the amount is increased it brings about the death of the organism. Sugar, on the contrary, is indispensable for the development of yeasts, but if the concentration is sufficiently increased it is inhibitory. All depends then, on the quantity. A substance is not an antiseptic of necessity; it acts, according to the amount, either as a food or as an antiseptic.

THE DEFENSE AGAINST POISONS

Substances harmful for bacteria have received the name of antiseptics. They must act by provoking physical modifications of the colloidal micellae which enter into the constitution of the cell, modifications leading to flocculations. The majority of antiseptics are active coagulants. We have seen, on the other hand, that traces of certain antiseptics, such as hydrocyanic acid, interfere with the catalytic action of metallic colloids.

The unicellular organism does not submit passively to the action of these substances, at least, not until the action becomes very violent. If the antiseptic is added to the medium in a quantity below the frankly harmful dose, and is then gradually augmented in amount, the microörganism reaches the point where

it can withstand doses, which if added all at once to the medium, would completely prevent development. It exercises its means of defense, it adapts itself and undergoes an evolutionary process.

The term antiseptic, applied to substances which in minute dosage are harmful to bacteria, is in reality a synonym for toxic, a term which is reserved to the same substances when they act upon higher organisms. Sublimate is an antiseptic for bacteria but is toxic for the animal, although fundamentally the action is the same. Man, who relates everything to himself, designates by different words identical phenomena according as the action upon him is favorable or the contrary.

Different toxic substances act in entirely distinct manners. Some attack all living cells uniformly. Others, on the contrary, possess an elective affinity for a certain class of cells, as atropine, the alkaloid of belladonna, which is fixed by certain bulbar cells. Indeed, all alkaloids show elective fixation. These substances are capable of acting in very small doses for they do not dissipate their action. When they have entered the circulation they remain there in a free state and are fixed to the receptive cells only as they are conducted to these cells by the circulating blood. The lethal dose of atropine, for example, is very small if it is compared to the weight of the man which it is able to intoxicate, being $\frac{1}{300,000}$ of the weight or thereabouts. But it is not distributed throughout the 60 or 70 kgm. of man, it combines only with the few grams of cells for which it possesses an affinity. This applies equally to the bacterial toxins, so that in reality the disproportion between the lethal dose and the weight of the substance killed is apparent only and is not as great as would appear at first sight.

The affinity which certain cells possess for a given substance is allied to the phenomenon of adsorption. This phenomenon is extremely complex. In the first chapter we have mentioned the processes of the adsorption of ions by the micellae. An explanation of the mechanism of this process would be too long and too technical to have a place in a text designed chiefly for biologists, who for the most part do not have occasion to acquire, or to retain, the mathematical knowledge to which it would be necessary to have recourse. But a simple example will suffice

to explain somewhat concerning the meaning of the term "coefficient of adsorption."

Let us dissolve in water a trace of picric acid, a trace so small that the solution will not appear to the naked eye to be colored. Into this solution let us introduce some silk fibres and some white strands of wool. After a few hours we will see that the wool fibres have adsorbed the coloring matter almost entirely; the amount which remains in the solution is not chemically detectable. The strands of wool are then stained a yellow color, although the solution had been so weakly tinted that its color was not visible. Furthermore, if we try to remove the color by repeated washing with large amounts of water we will find that the tint is fixed; that it resists washing. It is not the same with the silk. These fibres have remained white, or have acquired at most but a very faint yellow tint which does not withstand washing. We would say that the coefficient of adsorption of wool for picric acid is high, for silk it is low. The same experiment repeated with malachite green in the place of picric acid gives the reverse result; then the silk takes up the dye, the wool does not adsorb it.

The phenomena of adsorption are regulated by variations in the surface tension.

This example shows us the possibility of the unequal partition of substances introduced into the body, and the distribution of every substance occurs in proportion to the coefficient of adsorption of the cellular micellae for this substance. In the case, for example, of atropine, almost the entire amount is fixed to those cells which possess a high coefficient for this alkaloid. If none of the cells of the body possess a particular coefficient of adsorption the substance is distributed uniformly throughout all of the cells. If the coefficient of adsorption of all of the cells is negative no fixation occurs, and the substance is eliminated without having caused any disturbance.

It is essential to note that the true poisons, for which a group of cells possess a special adsorption coefficient, do not act in comparable dosage in different living beings. This is to be expected since their action depends upon an elective affinity for certain cells. Some of the alkaloids, for example, are toxic in very weak doses for certain vertebrates while others are able to withstand

considerable quantities without harm. It is thus that man is extremely sensitive to the action of nicotine, while the goat, on the contrary, is able to eat with impunity the leaves of tobacco, for which, indeed, it seems to have a particular liking.

An ingested substance does not necessarily penetrate into the body, for in animals provided with a digestive tract the interior of this tube is never within the body itself. The contents of the stomach or the intestine are in one sense inside of the animal but they are never within the body, properly speaking. In short, such an animal is comparable, schematically, to a hollow cylinder. If we take a tube of metal and place water in this tube the water is indeed within the cylinder but not in the tube, in the substance of the metal. It is not sufficient, then, that the poison be ingested to have it exercise its harmful action, it is necessary that it act, either on the walls of the digestive tract as is the case with a caustic substance, or that it traverse these walls and penetrate into the organism itself if it is to be toxic, strictly speaking.

The first defense against the majority of poisons harmful by ingestion consists in an effort of the organism to reject them, at least this is the case in animals in which this reaction is physiologically possible. Under the influence of the excitation produced by the toxic substance upon the sensitive terminations of the nerve fibres of the stomach, these fibres transmit the excitation to the "vomiting centre" which is located in the region of the medulla. The latter communicates with the motor nerve fibres of the muscles which contract the diaphragm, the abdominal wall, and the pylorus. Thus there is produced a simultaneous contraction of the whole, and at the same time there is a relaxation of the esophageal opening permitting the expulsion of the gastric contents. Let us note that this "defense" often works too late, as is the case for phalline, or does not work at all (aconitine, atropine, strychnine, etc.) or works needlessly, as in the vomiting caused by the first cigarette or by the injection of apomorphine. The same remark applies also to the intestinal disturbances caused by certain poisons.

The processes of elimination which are effected within the organism itself are quite important. Certain poisons are to be

found in native form in the saliva, the urine, the sweat, but the liver carries the chief burden in this type of defense. This organ, a true filter for the blood, arrests and stores the majority of toxins, alkaloids, alcohols, and mineral poisons. These last are eliminated gradually by the bile. The organic poisons undergo many transformations, either by oxidation or by hydration and decomposition, which lead to the formation of less toxic compounds.

The processes of transformation, and even of binding in native form, although exercised principally by the liver, are not carried on solely by this organ, for they may be performed to different degrees by all cells. Besredka has shown that particles of insoluble arsenic compounds are ingested by leucocytes.

Certain processes exercised by the cells of the organism present highly specialized characteristics and lead to reactions which it is impossible to predict. It is thus that, in the organism, hydrocyanic acid, the most effective of all the toxins, unites with sodium hyposulfite to give a sodium thiocyanate which is non-toxic.

At first sight one would perhaps attempt to attribute these phenomena of decomposition of toxic substances in the organism to a finalistic cause, having for its end the protection of life. This is not true. The toxic function of an organic compound, that is to say, the property which it possesses to act upon certain cells, is generally allied to a relative instability or to a complexity of the molecule. Oxidation or decomposition which results in transforming a compound into a more simple compound or into one more stable, are constant fermentative processes, and operate as well for substances which are toxic as for those which are not so. This is very obvious, for it is the exception for certain compounds to become, by oxidation, more toxic than the bodies from which they were derived. This is true for methyl alcohol in which oxidation gives formic acid, and this transformation is, indeed, brought about within the body. That which we call a defensive process, then becomes a process of death. The case of the union, mentioned above, between hydrocyanic acid and sodium hyposulfite should serve as a typical example of a finalistic defensive action, but, it never takes place naturally for the body never contains hyposulfite of sodium.

We have seen previously that bacteria accustom themselves to

support increasing amounts of antiseptics. The same thing occurs with organisms of more complex organization. This possibility of adaptation was recognized in antiquity. We know the legend of Mithridates, king of Pontus, who would attain protection from poisoning by habituating himself from youth to the action of all poisons then known. Such an adaptation, although it may not reach an absolute immunity, may lead to a progressive tolerance. This is in effect a defense by adaptation, which permits the body to withstand amounts of different toxins which are several times the lethal dose. Certain morphine users reach the point where they can stand a daily dose of 6 grams of morphine hydrochlorate, although a quantity one-thirtieth of this may cause death in a normal man. Adaptation to mineral poisons is likewise possible, as is shown by the example of certain peoples of Tyrol who habitually use arsenical preparations and maintain a perfect state of health despite the absorption of quantities which are several times the fatal dose.

OSMOTIC PRESSURE

The toxic crystalloids, since they possess an affinity for a special class of cells, form a particular case. But all crystalloids are in reality toxic, either because these substances introduced into the body are dissociated into ions which tend to replace the ions normally forming the micellar charges, provoking thus a cellular disorganization or, if it is a non-ionizable crystalloid, because this substance dissolved in the body fluids tends to break down the established osmotic equilibrium. This last action is exercised by all of the crystalloids, even by those which normally enter into the constitution of the living being.

To recall our ideas concerning osmotic tension will not be amiss, in view of the importance of this phenomenon in biology.

Let us take a glass tube closed at one end by a semi-permeable membrane, that is to say, by a membrane which will allow water to pass through but which intercepts the passage of all molecules of soluble bodies. Let us plunge this tube vertically, the membrane being on the bottom, to a depth of 10 cm. in a vessel containing pure water maintained at a constant level. Let us introduce into the tube a solution of a non-ionizable crystalloid (glucose,

for example) likewise to a height of 10 cm., in a manner to make the fluid within the tube on a level with that of the water in the outside container. It will be observed that the water will pass through the membrane and will dilute the solution inside, and as a result the volume of the solution increases and the level of the liquid within the tube becomes higher. It could be demonstrated, if one was able to work with tubes sufficiently long, that this rise would be 24 × 1033 cm. (that is to say, that the pressure on the membrane forming the bottom of the tube would be equal to 24 atmospheres) for all solutions, whatever they may be, containing a gram-molecule of crystalloid per liter, or in other words, containing in each liter a weight in grams representing the molecular weight of the crystalloid (for glucose, 180 grams). If we introduce into the tube, in the place of a non-ionizable crystalloid, an ionizable crystalloid (that is, a substance capable of dissociating into ions and which possesses by virtue of this property the additional property of transportation in an electrical current, as is the case, for example, with sodium chloride) one would have, in the place of the preceding height, always equal to 24 × 1033 cm. for a gram-molecule per liter, an increase in height always proportional to the ions existing in a free state in the liquid. It is known, on the other hand, that the ionization of a solution is the greater when the solution is the more dilute. The increase in pressure is then proportionally the more marked as the ionizable crystalloid becomes the more dilute.

Obviously, in practise, the measurement of osmotic pressure is effected by other more practical procedures, such as the lowering of the freezing point of a solution, which is proportionate to the osmotic pressure.

It is possible to compute the size of this osmotic pressure since it rises, as we have seen, to 24 atmospheres for a solution of glucose containing 180 grams of glucose per liter. For sodium chloride completely ionized in solution the pressure will be two times 24 atmospheres (there are two ions per molecule of sodium chloride, one of chlorine and one of sodium) or 48 atmospheres for a solution having 56 grams per liter, 56 being the molecular weight of sodium chloride.

It should be observed that we are not dealing with theoretical

figures. The pressure which will be exercised on the membrane of a cell containing a solution of glucose to the concentration of 180 grams per liter will be actually 24 atmospheres if this cell is immersed in pure water. It will be 2.4 atmospheres if the concentration is 18 grams per liter. It is of no moment how much of the solution is contained in the cell, the quantity may be only a thousandth of a cubic millimeter, but if the strength of the solution is 180 parts per liter, the pressure will be 24 atmospheres. To provide a balance for this internal pressure it is necessary to immerse the cell into a solution of equal osmotic pressure, for example into a solution of sodium chloride containing 28 grams per liter.

It is for this reason that if one introduces red blood cells into distilled water they rupture from the internal osmotic pressure. The hemoglobin diffuses instantaneously into the water. To have the red cell remain intact it is essential to have the liquid into which they are placed contain saline to the amount of 9 grams per liter. It may be readily calculated that the osmotic pressure of the interior of the cell is 8 atmospheres, since the osmotic pressure of the fluid with which there is an equilibrium is 8 atmospheres.

As is seen, the osmotic pressure communicated to a liquid by any given weight of crystalloid is inversely proportional to the size of the molecule. Furthermore, for the ionizable crystalloids this tension for a given weight is doubled, tripled, quadrupled, or quintupled according as the molecule of crystalloid yields 2, 3, 4, or 5 ions. For sodium chloride, with which the molecule of $NaCl$ gives a positive Na (sodium) ion and a negative Cl (chlorine) ion, the pressure is doubled. For potassium ferrocyanide, for example, it will be multiplied by 5, for $FeC_6N_6K_4$ gives a negative ion $(Fe(CN)_6)$ and 4 positive K ions when ionization is complete. If one deals with a mixed solution, composed of several crystalloids in solution, all of the pressures must be added.

REGULATION OF THE OSMOTIC PRESSURE

The tension or pressure of about 8 atmospheres which we have seen is that present in the cells of the organism is of the order found in a kettle due to the pressure of the steam. If pressures so great do not cause the bursting of all of the cells of the body it

is because the internal pressure is counterbalanced by the osmotic pressure of the fluid which bathes the cells.   But this equilibrium is decidedly instable.   Digestive absorption brings crystalloids and water into the blood, and on the other hand, the kidney, the skin, the intestine, and the lungs bring about an elimination of these substances.   The reactions of cellular life withdraw from the blood certain crystalloids (glucose, amino acids, for example) and introduce others (urea, etc.), and the influence which is exerted by these substances on the osmotic pressure varies with the substance, for they have very different molecular weights.   These are precisely the ruptures of equilibrium which are expressed by the differences in the osmotic pressure between the contents of the cells and the blood, and which intervene at the same time as variations in surface tension in the mechanism of exchange between these two media.   Water passes from the cells into the blood if the tension of the cells is higher than that of the blood, and in the opposite direction of the blood has the higher tension.

In glycogen the cell possesses a substance which permits the regulation of the internal osmotic pressure.   As is known, the carbohydrates, which form a portion of the energy-producing food requirements, are transformed by the ferments of the body into glycogen, which is a colloid, and this glycogen is again transformed into glucose for utilization.   The presence of glycogen, the colloid, does not have any appreciable influence upon the tension of the liquid in which it is found, but when transformed into glucose, which is a crystalloid, the tension of the cell increases in proportion to the weight of glucose formed by the action of the cellular ferments.

The organism possesses, in addition, means which permit the regulation of the osmotic tension of the blood.   When water or sodium chloride, the factors of most importance as regards tension, are in excess they are eliminated by the secretions, urine, sweat, and sometimes, but exceptionally, by intestinal flux. The organism maintains the tissues in equilibrium, and even in pathological conditions by effusion (hydrocele fluid, ascites) it pours off the temporary excess of water or dissolved material which has disturbed the equilibrium of the tension of its internal medium.

THE COLLOIDAL EQUILIBRIUM OF THE BLOOD

Not only for the purpose of maintaining an equilibrium of pressure must the blood have a constant composition. We have seen the importance of the composition of the intermicellar fluid upon the equilibrium of colloids. The slightest differences in composition, and especially in chemical reaction, suffice to disturb the colloidal equilibrium and to lead to a disorganization of the cell. The organism evolves reactions which permit it to maintain constant the composition of the blood.

The reaction of the blood is very slightly alkaline, it might be said invariably so. An infinitely small reduction in the titre of this weak alkalinity is enough to cause serious disturbance. Observation shows, in fact, that even during the course of diabetic coma preceding death the tendency toward acidity is so small that it can only be appreciated by means of extremely sensitive analytical processes.[2] But this alkalinity tends to vary continually. One of the end products of the processes of decomposition which furnish the energy requisite to the maintenance of life is carbonic acid. The cell eliminates this acid, it enters the blood, is fixed to the hemoglobin, and is transported to the pulmonary emunctory. It is unnecessary to discuss the mechanism of this elimination, as it is outside of our subject. But carbonic acid is not the only substance eliminated by the cells. The muscle cells always produce a certain quantity of lactic acid, resulting from an incomplete destruction of glucose. The cells of the liver produce normally a certain quantity of ketonic acids, which represent the physiological residues of the degradation of the fatty acids of the food. The presence in the blood of diverse acids, whose production by the cells is necessarily variable according to the more or less great activity of the cellular exchange at a given moment, is indeed, of a nature to vary the reaction of the blood, but observation shows that this reaction is practically invariable. What are the reactions utilized to maintain the fixity? The organism has at its disposal two means for this; physiological regulation and chemical regulation.

[2] The alkalinity of the blood of normal man is, upon a vegetable diet, $C_H = 0.38 \times 10^{-7}$; upon a meat diet, $C_H = 0.47 \times 10^{-7}$; in fatal diabetic coma, $C_H = 1.5 \times 10^{-7}$. C_H = concentration in H ions.

Pulmonary ventilation is regulated by the amount of H^+ (positive hydrogen ions) in the blood, originating chiefly from the ions derived from carbonic acid (H_2CO_3). When the pressure tends to become excessive by the arrival in the blood of diverse acids the respiratory centres are excited, the pulmonary ventilation is increased, and this leads to the elimination of larger amounts of carbonic acid, with a correlative diminution of the H^+ ions furnished from this acid. The blood maintains, then, its constancy in H^+ ions. If these H^+ ions are temporarily furnished by other acids there is an active elimination of carbonic acid, and therefore a reduction of the H^+ ions.

The chemical regulation has only recently been disclosed and is effected by an automatic process called "buffering." We will not enter into the details of this phenomenon since it would involve too lengthy explanations. These buffers, constituted in the blood principally by the carbonic acid and phosphoric acids and their sodium salts (sodium bicarbonate, mono- and di-sodium phosphates) and to a much lower degree by the albumins, possess at the same time the power of fixing both acids and bases. That is to say, the sum total of these bodies constitutes in the blood a system which gives it the power of a great reserve of H^+ and OH^- ions, which are liberated, through the arrival of either a base or an acid in the blood, thus maintaining automatically the fixed reaction of the tissue. We will see the importance of these facts to an understanding of the anaphylactic state.

CHAPTER II

The Defense Against Colloids: The Specific Fermentative Reaction

THE DEFENSE AGAINST FOOD COLLOIDS

The "defense" of the organism against colloids has been, throughout the last thirty years, the object of a very considerable number of investigations.

The introduction into the body of a foreign colloid, and in the present case we will consider only the albuminous colloids, may be effected in four different ways: (1) by transfer through the intestinal barrier, (2) by the development of bacterial colloids within the body during infectious disease, (3) by the resorption of tissues as the result of traumatism, and (4) by experimental introduction by injection.

The specific reactions provoked by the introduction of a foreign albumin into the body are of two different types, produced simultaneously; first, digestive reactions of ferment nature which tend toward the maintenance of the organic equilibrium, second, reactions of a purely colloidal nature, non-fermentative, which tend toward the rupture of this equilibrium.

Plants, obtaining from the inorganic compounds the elements which serve for the building up of their tissues, are not exposed naturally to conflict with foreign colloids.

With the protozoa the conflict is constant, but as the colloids which serve as foods are ordinarily derived from other living beings, their reactions are reduced largely to a defense against bacteria.

In animals provided with a digestive tract foreign colloids do not *normally* gain entrance to the interior of the body. To avoid complicated discussion of the reactions in animals of this type, let us consider only the case of the vertebrate, that of man in particular.

All foods are constituted, almost in their entirety, of colloids of either animal or vegetable origin. Normally these colloids,

in their native form, can not pass through the walls of the digestive tract. They are first altered by coagulative processes in the stomach through the action of the hydrochloric acid or the digestive juices, according as they are albumins or casein. The first act of digestion is thus a reaction of defense against the colloids. As long as a substance is in a colloidal state it is formed of micellae which bear the mark of their origin; the substance is still "living." Once coagulation is effected, the micellae are destroyed, the mark of their origin disappears, they are transformed into a neutral substance and are "dead." Upon this dead substance the digestive ferments act, and the complex albumins among the other substances are step by step reduced to the form of crystallizable amino acids, which entering the intestinal mucosa penetrate into the organism.

THE DEFENSE OF THE ORGANISM AGAINST ITS OWN FERMENTS

Before considering the specific reactions induced by the introduction of a foreign colloid, it may be well to see how the organism reacts to its own ferments.

The destruction of albumins, that is, of foreign cells, in their transformation through degradation to crystalloids, is effected by ferments secreted by the cells of the organism. How does it happen that these ferments, which decompose the substance of foreign cells, do not attack the body cells which produce them, or that, once liberated in the digestive tract, they do not deteriorate the walls? We will see that if this damage does not take place it is simply because of a defense.

In the first place living colloid matter appears refractory to the action of the digestive ferments secreted by the cells of animals; indeed, the ferments may even be destroyed by certain of these colloidal substances. This is not surprising for the ferments are in colloidal state. Thus we have two colloids together which react one upon the other. As long as the material remains in a colloidal state it is "living" and defends itself, but by a wholly physical process however, such as are all defensive phenomena. It is well known that raw egg white will destroy papaine, that the same egg white paralyzes the action of pepsin and of trypsin, and that, on the other hand, neither pepsin nor trypsin are able to exert any action whatever upon a colloidal albumin although they attack it energetically once it is coagulated. But

coagulation destroys the colloidal state, "kills" the colloid, and that which remains after coagulation is dead matter.

The body is confronted by a two-fold problem; the protection of the albumin of its own cells against the action of the ferments, and, particularly, the protection of the ferments essential to digestive processes against the colloids of the tissues. This question of reciprocal protection the body resolves in a most ingenious fashion. The ferments possessing an action for these albumins are not formed intact by a single cell, as we will see. It is quite remarkable that only such ferments as possess an action against these albumins, compounds whose nature approaches that of the albumins of the body cells, are produced in a round-about way, so to speak. All of the others ferments which act either upon an albumin, such as casein, which is not normally present in the body cells, or upon the carbohydrates are secreted in a form quite ready for action. The cell has nothing to fear from ferments which act upon substances which the cell does not contain.

Pepsin is not produced by the cells of the fundus glands of the gastric mucosa; these cells secrete a propepsin, completely inert. This propepsin is probably only the nucleus of the future pepsin micella, it lacks the element essential for action, its charge of negative ions, which is provided for it immediately when it comes in contact with the hydrochloric acid secreted by the cells of the lining of these glands.

The formation of trypsin is still more complicated. The cells of the pancreatic gland produce several ferments, a lipase, an amylase, and a protrypsin. The first two, acting respectively upon the fats and the starches, can have no effect upon the cellular contents. They are secreted preformed, ready to exert their action. The protrypsin is, on the contrary, inactive.

On the other hand certain cells of the mucosa of the duodenal region and of the upper jejunum secrete (stimulated by the secretin), a colloidal substance, the enterokinase. The pancreatic juice is poured into the duodenum by the pancreatic duct, the protrypsin which it contains is transformed by contact with the enterokinase which it meets there, into a ferment, trypsin. It is not quite exact to say that protrypsin is transformed into trypsin, any

more than to say that propepsin is changed to pepsin. The union of protrypsin with enterokinase, neither of which is a ferment, produces trypsin, just as the union of propepsin with the activating ions of HCl yields pepsin.

What is the active element supplied by the enterokinase? In all probability it is the positive calcium ion. In fact, it is possible to activate, experimentally, a protrypsin by means of a calcium salt, as has been shown by Délezenne.

The ferments which transform albuminous materials, whose composition approaches that of the substance of the cells, are not found, then, at any time in active form within the interior of a cell. One group of cells furnish the granule, another the ions, but the active micellar complex is "born" in the digestive tract.

But when once formed, why is not the ferment able to act upon the walls of the digestive tract? We have seen in Chapter I that a gel acting upon a sol usually leads to a dissemination. Among the gels, there is one, however, which provokes a contrary reaction, and this is mucin. And it is interesting that mucin is secreted precisely by the cells which line the digestive tract, thus, by contact with these cells the ferment must be inactivated, flocculated, and in this form it is unable to react upon albuminous substances. The secretion of mucin constitutes an act of auto-defense.

PROTEIN SHOCK

We have seen that under normal conditions none of the food colloids penetrate the intestinal wall until after they are broken down and are thus deprived of their living character, their individuality. The digestive ferments change them to the crystalloid state. Sometimes however, as the result of abnormal circumstances a food colloid may gain entrance to the interior of the body.

It can be experimentally demonstrated that the ingestion of large quantities of foreign albumins—raw egg white, serum, raw meat—causes a derangement of the digestive functions with the result that small amounts of these colloids, in native form, gain entrance to the circulation. This intrusion results in a rupture of the colloidal equilibrium of the blood, and is accompanied

by a symptom-complex to which has been given the name, protein shock.

Experimentally, this shock has been intensively studied, not so much by inducing the passage of native albumin into the circulation by way of the intestinal route, since this is difficult to accomplish, as by injecting the foreign substance directly into the body.

It has been rather more than thirty years since physiologists first began to study the effect of the intravenous injection of foreign substances upon coagulation of the blood. During these studies they observed that among the injected substances the organ extracts, the peptones, and in general albuminous substances, provoked special disturbances, and these reactions were designated as peptone shock or protein shock. In 1898 Délezenne showed that identical effects upon the blood followed the injection of any albuminous substance whatever. Later, Arthus showed that this similarity in effect quite naturally resulted from a similarity in cause. It is not the peptone, the ovalbumin, or even the venom of the snake, as a chemical compound which acts; they act rather as colloidal substances. Protein shock manifests itself chiefly in a sudden lowering of the blood pressure, and this is accompanied by a decrease in the coagulability of the blood. It is needless to consider this further at the moment, for we will find this same shock later when we have occasion to discuss a much more violent form associated with repeated injections of a protein.

The massive injection of certain colloidal substances, of albumins in particular, is sometimes followed by its appearance, as native albumin, in the urine. This may be observed in man who has received an injection of horse serum. The first reaction of defense consists, then, in an elimination of the native substance, but this elimination is far from being complete, and does not invariably occur.

Unless the quantity of injected albuminous substance is very great the shock provoked by a single injection does not endanger the life of the individual. Sometimes delayed disturbances may be observed which, very often, are much more obvious than was the shock itself, indeed, the shock may have passed unobserved. These delayed reactions have been best studied in man in con-

nection with the foreign albumins,—the serum albumins and serum globulins of therapeutic sera (anti-diphtheritic, anti-tetanic, etc.), injected for curative or preventive purposes. These disturbances represent that which von Pirquet and Schick have termed "serum disease." It should be noted that the antitoxic properties of such sera have nothing whatever to do with the manifestation of such reactions. Serum disease is induced by albumins only, and may be frequently observed following the administration of normal horse serum.

Not all individuals are equally susceptible; serum disease manifests itself in only about fifteen per cent of individuals following a *first* injection. In these, eight to ten days after the injection, an exanthem of greater of less extent in the form of an urticaria appears. In some cases the whole surface of the body is covered. The lymph nodes of the region near the point of inoculation become hypertrophied. And, accompanying these reactions there is usually fever. All of these symptoms persist for several days and then they quickly disappear, leaving no trace. Only exceptionally, and accidentally does serum disease assume a grave form. Later we will discuss the cause of this delay of eight to ten days between the injection of the albumin and the onset of the disturbance.

FERMENTS OF DEFENSE

We have seen that, at times, the first defense employed by the organism is the elimination of the native albumin through the urine.

The second defense is general, it consists in the production of "protective ferments" (Abderhalden) which appear in the blood in about 4 days after the injection. These ferments lead to a true intravascular digestion of the foreign protein.[1] Normally, the blood contains ferments of defense which act upon the non-protein colloids and upon the fats. The introduction of these substances by a parenteral route[2] does not lead to the pro-

[1] Délezenne was the first to show that after the injection of gelatin into the circulation the blood acquired the property of peptonizing this substance.

[2] That is, by intravenous injection, or by injection under the skin, into the peritoneum, the pleurae, or into the tissues.

duction of a specific amylase, or lipase, as several authors have suggested, but to a simple increase in the titre of ferments normally present in the blood.

In general, experiment shows that the blood of a normal animal does not break down any of the albuminous substances, but if similar tests are made some days after the introduction into the animal by a parenteral route of some definite albumin, it will be seen that the processes of disintegration are operative. The ferment produced is not strictly specific, indeed, if one injects, for example, egg white, the serum acquires the property of degrading a number of complex albuminous substances, and even their products, such as the peptones, are fractioned.

These ferments of defense disappear from the blood after about twenty days. A second injection of the albumin, given after the complete disappearance of the fermentative power of the blood, leads to their appearance much more quickly than was the case after the primary injection. Furthermore, these reacting bodies seem to be more specific.

We have seen that an albumin ingested in very large quantities "forces" the intestinal mucosa; a portion of the native albumin passes through, and it is known that albumin gaining entrance to the body in this manner causes the production of defensive ferments just as though it had been injected directly into the circulation. This shows that the formation of these ferments is not purely an experimental phenomenon, but that it may likewise occur naturally.

The different tissues of an animal are formed of cells which are more highly differentiated the more complex the organization of the animal. Consequently, it is to be expected that the intravenous injection of one animal with organ preparations of another species would lead to the elaboration of defense ferments, and this is, in fact, what takes place. Shortly after such an injection it can be shown that the blood contains one of these ferments[3] which

[3] Many authors hold to the view that here there is the formation of a specific ferment, that is, a ferment possessing the power of degrading only an albumin identical with that which has been injected, and that this formation is accompanied by the production of several other ferments each attacking a related albumin. The hypothesis assuming the

attacks a number of proteins, provided they are derived from a tissue similar to that which had been injected. For example, a rabbit is injected intraperitoneally with 2 grams of macerated rabbit brain. Five days later it can be demonstrated that the serum of this rabbit degrades the nucleoproteins of the brain of the rabbit, of man, of the calf, etc., but it is without action upon the nucleoproteins of other organs. Injections of any tissue whatsoever give comparable results.

Resorption of tissues in an animal provokes the appearance, in the same animal, of defensive ferments. This fact is of great importance, for such ferments, or processes of defense, often have occasion to function. Indeed, they must act every time that there is damage to a tissue, a wound, a bruise, or a burn of any part of the body. If one produces in a rabbit a large lesion of the muscle it can be shown that after four or five days the blood of this animal contains a ferment which reacts upon muscle, whether it be of the rabbit, the calf, or the dog, but which is inert when combined with renal or hepatic tissue of any animal.

As a further example, Abderhalden and Schiff state that the serum of a man who had suffered a few days before a considerable bruise of the muscles attacked the proteins of muscle tissue but was without action upon proteins derived from other organs. Thus, it appears that the defense ferments show an organ specificity rather than a species specificity.

The mechanism of the formation of these ferments of defense is not as yet understood, nor do we know in what organs they originate. Indeed, are they substances or are they properties? It is impossible to say.

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production of a single *non-specific* ferment should not be rejected a priori, for it is much more readily understood. However, proof of the correctness of the one or the other of these hypotheses is impossible, for the ferments can not be isolated; their action can only be demonstrated "en bloc."

CHAPTER III

THE REACTION AGAINST COLLOIDS: THE PHENOMENA OF FLOCCULATION

THE ANTIGEN

Let us pass now to examine a second phase of the phenomenon of the defense against colloids, a phase infinitely more complicated, and involving specific reactions, non-fermentative in character.

The production of defense ferments is accompanied by the simultaneous development of those substances which have been termed antibodies. While the defense ferments do not appear in the blood, or at least are not revealed by our methods of determination, unless a relatively large quantity of protein has been injected, the antibodies appear as the result of the introduction of infinitely small amounts of foreign proteins.

In the following discussion we will adopt the terminology usually employed, designating as antigen (that is to say, the generator of anti(body)—generator of antibody) all substances capable of provoking in the organism the formation of antibodies. It has been discovered that all protein substances, and protein substances only, that is, albumins, can function as antigens. To be antigenic this albumin must, moreover, be found in a colloidal state and be derived from an animal different from that in which it is to function as antigen. Human serum, injected into another man does not lead to the formation of antibodies. Horse serum injected into man does cause antibody formation. This last is, therefore, antigenic in man.

The heterologous albumins which at one time or another may be found within the body of an animal may be of very diverse origins. Sometimes they may be derived from the food, as we have seen, but there is another more important source of these foreign substances within the body, namely, the elaboration of foreign materials during the course of bacterial infection. But in this connection it is necessary to be guarded lest a serious error

be committed, that of confounding the reactions of the body to the bacterium as a banal protein substance with reactions, fundamentally different, associated with the pathogenic bacterium in its character of a living parasitic being. All of the errors which weigh heavy upon the study of immunity have been derived, as we will see, from this confusion. This distinction is the more necessary since the reactions, purely physiological in character, contribute nothing whatever to the defense of the body against the pathogenic bacterium. Indeed, they may be injurious. We will see, in fact, that the two types of phenomena lead to exactly opposed results, and that those which are based upon the physiological reaction appear finally as the antithesis of immunity.

For the moment we will not consider the question of the defense against the bacterium as a living being; that subject is reserved for special discussion later. We will only deal here with the reactions of the body against the bacterial protein, to which it reacts as it would to any protein whatsoever, as serum or egg white.

THE PRECIPITINS

Krauss (in 1897) showed that a bouillon culture of the cholera vibrio filtered through a candle, that is, freed of the bacterial bodies and perfectly limpid, became opalescent when mixed with a few drops of serum derived from an animal prepared by injections of the cholera vibrio. Little by little the opalescence condensed in floccules which fell to the bottom of the tube. He repeated the same experiment, adding to filtered cultures of typhoid bacilli, or plague bacilli, the sera of animals prepared by injections of *B. typhosus*, or *B. pestis*, and he noted in the corresponding filtrates the appearance of floccules. Two years later Tchistovitch, having prepared rabbits by repeated injections of minimal quantities of eel serum, noted that if he added eel serum to definite amounts of the sera of these rabbits a turbidity quickly manifested itself due to the appearance in the mixed fluids of very fine agglutinates which later condensed into voluminous floccules falling to the bottom of the tube. The blood of normal rabbits did not possess this property, indicating that the reactive capacity is developed by the injections of the eel serum. The serum of the eel functions as antigen and induces the formation of antibody—precipitin.

At about the same time Bordet likewise observed that the serum of a rabbit prepared by injections of chicken blood possessed the property of flocculating the chicken serum which he added.

It has since been proved that this is a general law; the body of any animal whatever receiving an injection of any albuminous material whatsoever responds by the formation of specific precipitins; the serum possesses the property of flocculating a pseudo-colloidal solution of the albumin injected. The result is the same whether the albumin be derived from a serum, from normal or pathologic transudates, or from animal, vegetable, or bacterial cells. There is but one single condition to the appearance of precipitin, namely, that the albumin injected be heterologous with regard to the albumins constituting the tissues of the animal which receives the injection. If one injects a rabbit, for example, with the serum of another rabbit, the serum of the first does not acquire to any perceptible degree the property of flocculating rabbit serum.

Is precipitin a substance? This is hardly probable. It is much more likely that the flocculation is due to a property of the colloids of the serum, and results from a modification of the colloidal equilibrium brought about by the presence of a foreign colloid. However this may be, neither of these hypotheses can be confirmed in the present state of our knowledge. We see a precipitation, a flocculation without knowing how, in the last analysis, it is provoked. For convenience in discussion of the facts we will say that a precipitin is formed, without elaborating any hypothesis as to its fundamental nature, whether it be a substance or whether it be a property.

THE FORMATION OF PRECIPITINS

Precipitin appears in the serum of an animal in whose body there occurs a breaking down, either natural or experimental, of a foreign albumin. Precipitin can be detected within four or five days after the introduction of the albumin and it increases in quantity up to the twelfth or fifteenth day. From that time on the amount diminishes although its presence may still be demonstrated for several months.

The serum of an animal prepared by a single injection of an albumin is, in general, relatively poor in precipitin. It is necessary to add several drops of the colloidal solution of the corresponding albumin to obtain opalescence. If the injections are repeated the precipitating power augments considerably, so that after three injections but a minute quantity is sufficient to cause flocculation. The precipitating power reaches its maximum about twelve days after the last injection, and may attain a considerable degree of intensity; a millionth of a cubic centimeter of serum from a rabbit prepared by injections of a vegetable albumin, introduced into a cubic centimeter of this albumin, may produce a very definite turbidity.

Let us repeat that in all cases the precipitin formed is strictly specific. Flocculation is produced only if one mixes the serum of an animal prepared by injections of a definite albumin with a solution of this same albumin.

PARENTAL RELATIONSHIPS OF ANIMAL SPECIES

It is clear in view of the conditions which govern the formation of precipitins that many advantages may be derived from the phenomenon. Can it serve to determine the parental relationship of two animal species? For this it is only necessary to see if the precipitin formed under the influence of injections of an albumin derived from the body of one of these species flocculates likewise the corresponding albumin derived from the other. The same end may be attained by determining if a precipitin will appear in an individual of the first species by the injection of an albumin derived from the body of an individual belonging to the second.

For such experiments serum albumins are chiefly employed. Let us inject, for example, human serum into a rabbit. There is formed in the blood of the rabbit a precipitin which flocculates human serum. But it likewise flocculates, and with the same intensity, the serum of the higher monkeys, it flocculates the serum of the lower monkeys but weakly, and it does not react at all with the serum of any other animal species whatever. Let us inject a man with horse serum. There is the formation of a precipitin flocculating horse serum and that of the donkey. But if we inject a man with the serum of a monkey, such as the gorilla,

the chimpanzee, or the orang-outang, no precipitin appears. We obtain just the same result as if we inject the serum of another man. Such experiments allow us to conclude the close ancestry of man and the anthropoid apes.

DIAGNOSTIC APPLICATIONS

In a bacterial infection the albuminoid substances of the bacterial bodies necessarily become diffused throughout the body. Thus, the blood of an animal which has been affected with an infectious disease for a time sufficiently long to allow of the formation of precipitins ought to flocculate filtered cultures of the bacterium, the cause of the infection. And this, indeed, is what experiment demonstrates.

In a septicemic infectious disease, that is to say, in one in which the bacterium invades the circulation and as a result all of the organs, the bacterial albumins ought to be distributed throughout all of the tissues, and a filtered maceration of any organ whatever ought to flocculate in the presence of a serum prepared by injections of this bacterium. This is likewise what experiment shows. This fact has been utilized for the retrospective diagnosis of certain diseases, as, for example, anthrax. Let us suppose that an animal has died on a farm. The veterinary should determine if the death was in fact due to anthrax, for in such a case it is imperative to vaccinate all of the animals still alive. It is sufficient to prepare an extract of an organ of the dead animal, to filter this extract to procure a limpid fluid, and to add it to a serum derived from an animal prepared by the injection of cultures of *B. anthracis*. If the animal died of anthrax the albumins of the anthrax bacillus contained in the filtered liquid will flocculate under the influence of the precipitin contained in the serum of an animal prepared by injections of this same albumin. The presence, or absence, of flocculation indicates, then, whether the death of the animal was due to anthrax or to some other disease.

THE FLOCCULATION OF BACTERIA

For some years before the discovery of the precipitins it had been known that bacteria suspended in a liquid could be flocculated

by the action of the serum of an animal prepared by injections of this bacterium. The phenomenon was, however, interpreted wrongly as an act of defense on the part of the body against the bacterium. This hypothesis, contradicted by the facts, has been abandoned.

The flocculation of bacteria under the influence of precipitin had been designated by the name of agglutination, and the precipitin involved was termed agglutinin. Let us conserve the terms established by usage, but let us remember that the word flocculation, the only exact term, is replaced by that of precipitation when dealing with an albumin in solution, and by agglutination when the material flocculated is particulate, such as bacterial bodies. The hypothetical substance provoking the phenomenon is called precipitin in the first case, and agglutinin in the second, although in all cases the same principle is operative.

Charrin and Roger, in 1889, noted that if one inoculated *B. pyocyaneus* into normal rabbit serum a culture of the bacillus is obtained which has an uniform turbidity, but if, on the contrary, the inoculation is made into the serum of a rabbit "prepared" by a series of injections of minimal quantities of the same pyocyaneus organism the culture in the serum of this animal is found only at the bottom of the tube, in the form of small masses, while the supernatant liquid remains perfectly clear. Several years later Bordet observed that if one added to a culture of cholera vibrios already well developed or to a suspension of these vibrios in saline a trace of the serum from a rabbit prepared by injections of cholera vibrios the phenomenon observed by Charrin and Roger was reproduced; the vibrios adhered to each other forming small masses which fell to the bottom of the tube.

Gruber and Durham next showed that this is a general phenomenon. To the injection of any bacterium whatever the body responds by the formation of an agglutinin which possesses the property of inciting flocculation, that is, agglutination of the bacteria of the same species as those which had been injected into the experimental animal. The agglutinin formed is specific. The serum of an animal prepared, for example, by injections of the cholera vibrio agglutinates the cholera vibrio and nothing but the cholera vibrio, remaining without action on other bacteria. The

serum of an animal prepared by injections of dysentery bacilli agglutinates the dysentery bacillus and nothing but dysentery organisms.

The specificity is strict but not absolute. There are, for example, three varieties of dysentery bacilli. The serum of an animal prepared with one of these varieties agglutinates strongly the type with which the animal has been injected, and agglutinates, although more weakly, the other two races.

There are many species of bacteria and the different races show a great variability in their susceptibility to agglutination by specific sera. With certain ones, as typhoid bacilli, cholera vibrios, and the dysentery organisms, the flocculation can be produced if one adds to 10 cc. of culture as little as 0.0001 cc. of the serum taken from an animal prepared by injections of these organisms. With other organisms the quantity of serum must be appreciably increased.

The formation of specific agglutinin is not purely an experimental procedure. Each time that a bacterium invades the tissues, whether it has been introduced voluntarily by means of an injection, or whether it has grown there naturally, as is the case in infectious disease, the serum acquires the property of provoking the flocculation of bacteria of the same species.

DIAGNOSTIC DETERMINATION OF BACTERIAL SPECIES

In view of the above facts it can be readily understood that bacteriologists have made application of the phenomenon of agglutination for various purposes. It may be employed to make a diagnosis of an infectious disease. The agglutinating property of serum appears within five to eight days after the entrance of the bacteria into the circulation. For diagnosis it is sufficient to see if the serum of the patient agglutinates a culture or a suspension in saline of the bacterial agent supposed to be the cause of the disease. Having given, for example, a disease which presents clinical symptoms resembling typhoid fever, a small amount of blood is taken from the patient, the blood is allowed to coagulate, and of the serum so obtained a hundredth of a cubic centimeter is introduced into a cubic centimeter of a sus-

pension of typhoid bacilli. If there is an agglutination of typhoid bacilli the clinical diagnosis is confirmed. To Widal we owe this method of sero-diagnosis.

The specificity of the agglutinins allows us to solve another problem. There are many species of bacteria which are difficult to distinguish one from another and the agglutination reaction allows us to establish their true relationship. We may have at hand a culture of a bacterium which presents the morphological characteristics of the cholera vibrio, but there are other similar vibrios which do not produce cholera. Can we tell one from another? Prepare a rabbit by two or three injections of a culture of the true cholera vibrio. Twelve days after the last injection withdraw some blood from the rabbit. Take then a culture of the vibrio the nature of which is in question and add to it a small quantity of the serum from the prepared rabbit which contains an agglutinin specific for the true cholera vibrio. If agglutination takes place the nature of the doubtful organism is established, it is a cholera vibrio. Otherwise it is not.

Bacteria are not the only elements which agglutinate through the action of a specific serum. The red blood cells agglutinate in the same manner, and here again there is a specificity of action. A rabbit which has received injections of sheep cells yields a serum which agglutinates sheep cells and only sheep cells. It can be shown that the agglutinin forms under the action of the albumins of the stroma, that is, the skeleton of the red cell; the hemoglobin contributes nothing to the phenomenon.

It is unnecessary to discuss the aid that this last phenomenon brings to legal medicine, since it is evident that the reaction renders it possible to determine the nature of a blood; it enables us to determine the species of animal from which the blood came that is found upon blood-stained materials.

THE CAUSES AND EFFECTS OF AGGLUTINATION

The agglutination of bacteria under the influence of that which is termed agglutinin, and which is in reality confounded with precipitin, is what might be termed an artificial phenomenon. It occurs in vitro only. Within the body bacteria are not flocculated to any appreciable extent. One may inject an animal

previously inoculated with bacteria with a large quantity of any type of an agglutinating serum, even ten times or one hundred times the quantity which would produce in vitro the flocculation of these bacteria if they were suspended in a volume of saline equal to the weight of the animal and no obvious agglutination takes place in vivo. One might perhaps object that it may not be necessary for the bacteria to undergo an agglutination for the agglutinin to play a defensive rôle, for this agglutination is only the second phase of the process which begins by an elective fixation of the agglutinin to the bacterial body. That which is important, it may be said, is not the flocculation, which is an accessory phenomenon, but it may be the fact that the bacterium has fixed agglutinin and this may modify its properties. Experiment makes reply to this. The vitality of agglutinated bacteria is not impaired. Bacteria pathogenic for a given species of animal remain pathogenic, and to the same degree. Agglutinated bacteria transplanted upon an appropriate culture medium reproduce as do normal bacteria. It can be shown experimentally, as we will see, that they become even more resistant than normal organisms to certain destructive actions. If one adds to a culture medium an amount of specific serum an hundred times, or even a thousand times more than sufficient to cause agglutination and then adds some of the bacteria sensitive to the agglutinin contained in the serum, the bacteria grow, they develop as actively as they would in plain bouillon, they kill the experimental animal in the same dosage as do normal bacteria of the same species, yet they grow only in the form of an agglutinate.

The formation of an agglutinin does not constitute, then, a true specific reaction of the organism against the bacterium. The agglutinin is a principle reacting against the albumin of the bacterium. And it is directed only upon this albumin in solution. It remains without action upon the bacterium itself, since the vitality or the properties of the organism are in no way modified. The injection of animals with disintegrated bacteria, in solution— provided the albuminous substances remain in a colloidal state— produces the same effect as to agglutinin formation as does the injection of living intact bacteria. The reaction represents a general law; the reaction induced by the intrusion of a foreign

albumin tends to disturb the colloidal equilibrium of the blood, and the "defense" consists in a reaction of the colloids of the blood with the heterologous colloids introduced. The bacterium, in so far as it is a living being, plays no part in the phenomenon.

THE MECHANISM OF FLOCCULATION

Analysis of the phenomenon shows that it is indeed a colloidal reaction. Let us take, as has Bordet, a culture of typhoid bacilli and centrifugalize them. The bacilli collect in the bottom of the tube in the form of a sediment and the supernatant liquid is perfectly clear. Take the sediment, wash it with distilled water, and collect the bacilli a second time by centrifugalization. Suspend the sedimented, washed bacilli in distilled water and add to this suspension some serum which specifically flocculates the typhoid bacillus. No agglutination takes place. Add now to the suspension a trace of ordinary salt, sodium chloride. Agglutination occurs. In an experiment of this type the sodium chloride may be replaced by any electrolyte.

For the reaction precipitin is even unnecessary; the flocculation of bacteria can be effected by means of electrolytes alone, especially with the salts of the heavy metals. The inorganic colloids, silica, or the colloidal metals in very small amounts likewise cause agglutination. But this action is not specific; all bacteria are agglutinated and without distinction as to species. Precipitin contributes, then, the factor of specificity. That which flocculates is not the albumin it is the albumin-precipitin complex, and this complex absorbs the ions provided by the electrolyte.

In Chapter I we have considered the phenomena of the flocculation of colloids under the influence, either of electrolytes or of other colloids. The similarity of those phenomena with that of the precipitation of albumins by a specific serum indicates that the latter is a reaction of the colloidal state. The question is somewhat complicated by the fact that the intact bacterial bodies are flocculated. In reality, this flocculation is a secondary phenomenon. We have seen that the medium filtered free of bacterial bodies contains bacterial albumins in solution, and that these flocculate when reacted upon by precipitating serum. The same reaction evidently takes place in the unfiltered culture, in

which there occurs, simultaneously, a flocculation of the albumins secreted by the bacteria and an agglutination of the same bacteria. This second phenomenon is certainly under the domination of the first.

THE SPECIFICITY OF PRECIPITINS

It has actually been demonstrated that the phenomenon of precipitation is associated with the colloidal state of the substances which provoke and which undergo the flocculation. But all colloids are not antigens, they do not all produce the formation of an antibody. Functioning as antigens are the albumins of bacteria, of animals, and of vegetables. We must make an exception for gelatin and for peptone, since these substances do not show a sufficient "individuality." Peptone is, moreover, already a split-product of the albumins. The individuality of the protein colloid is, in fact, an indispensable condition for its functioning as an antigen, and the production of an antibody in a given animal occurs only if the colloid injected differs from the colloids normally present in this organism.

It appears that the antibodies are formed only on the condition that the colloid which penetrates into the circulation of an animal possesses a chemical composition analogous to that of the albumins which are found normally, or which may be found, in the blood of this animal, although in a different physical state.

It may be recalled that a micella is formed of a granule carrying an electric charge represented by ions. To be able to function as an antigen a colloid seems to require that it be formed of micellae containing a granule of a chemical composition analogous to that of the granules pertaining to some of the micellae which appear, or which may appear, at any time in the circulating blood. But the charge of these micellae, represented by the adsorbed ions, must be different from that of the corresponding micellae of the blood. And this is the case for the albumins, and for albumins only.

CHAPTER IV

The Reactions Against Colloids: The Artificial Phenomenon of Fixation

THE GRANULAR TRANSFORMATION OF VIBRIOS

We have seen that the penetration of the body of an animal by a foreign albumin results in the appearance of antibodies in the blood of this animal. These antibodies possess the property of flocculating an albumin of the same nature as that which led to the formation of the reacting antibodies. The flocculating antibody is most probably not a substance, but is rather a property resulting from a state of colloidal equilibrium of the blood plasma.

The formation of flocculating antibody is accompanied by the appearance of a second property, or of a second antibody, termed sensibilisatrice, fixateur, sensitizer, or amboceptor. Up to the present time this second property has quite generally been distinguished from the first, although probably but a single and unique property is operative; the flocculating antibody and the sensitizing antibody both resulting from the same state of equilibrium of the blood, induced by the penetration of an heterologous albumin into the body.

The facts involved are extremely complex, and are the more difficult of comprehension since they have been discovered little by little, and since each step in advance has been accompanied by a theory which, most often, has denatured the facts in such a manner as to make them fit into the hypothesis. In order to facilitate the discussion let us adopt, in presenting them, the chronological order of their discovery.

Let us prepare, as did Pfeiffer, a guinea-pig by two or three subcutaneous injections of the cholera vibrio. About twelve days after the last injection inoculate into the peritoneum of this guinea-pig some living cholera vibrios. After a few minutes remove a drop of the peritoneal exudate. The microscope shows that the

vibrios, which normally present the appearance of small curved rods, are transformed into granules. The same result can be attained by preparing the guinea-pig by injections of albumin extracted from the vibrio.

Shortly after this observation Metchnikoff showed that this phenomenon could be reproduced in vitro. It is sufficient to add to a suspension of cholera vibrios a drop of the peritoneal exudate from a prepared guinea-pig. Bordet next discovered that he could obtain the same result by substituting for the peritoneal exudate the serum of the prepared animal, and this last author was finally able to establish the conditions governing the manifestation of the phenomenon.

Select a serum which, when fresh, brings about the granular transformation, and repeat the above experiment, with the same serum, some days later. It will be found that the old serum has lost its power of transforming the vibrios. Or, as a variant to this experiment, add a few drops of a suspension of the vibrios to the serum of a prepared animal after this serum has become inactive through ageing. And, in another tube, add to a suspension of the same vibrios some fresh serum from a normal, not prepared, guinea-pig. No transformation takes place in either of the suspensions. Unite the two. The granular transformation follows. Again, we may repeat the same experiment, but in place of allowing the serum of the prepared animal to age for several days, we heat it at 56°C. It has been rendered inert, but it can be reactivated by a fresh normal serum.

The conclusion is obvious. The granular transformation takes place through the intervention of two factors. The first of these, A, exists only in the serum of an animal prepared by injections of the vibrios. It resists ageing and likewise a temperature of 56°C., but experiment shows that it is destroyed at about 65°C. It is, then, relatively stable. The second factor, B, exists normally in the serum of animals, prepared or not, and is destroyed by ageing and by a slight increase in temperature. It is thus less stable than the first. It is labile.

The next step in our knowledge of the subject followed when it was shown that it is this B factor, present in all normal sera, which effects the granular transformation, but that the B factor is able

to attach itself to the vibrios only if these last have been previously treated with the A factor, which is specific and is present in the serum of prepared animals only. It is for this reason that the A factor has been named sensibilisatrice, or sensitizer. The B factor, which is the alexin of Buchner, is the complement.

A long controversy ensued, principally between Bordet and Ehrlich, on the subject of the mode of union between the antigen, the sensitizer, and the complement. For Ehrlich it is a purely chemical phenomenon. The molecule of the sensitizer possesses, according to him, two radicles, the one having an affinity for the bacterium or the cell, the other for the complement. The first union is not able to "engage" unless the cell or the bacterium is of the same nature as the antigen. The other union "engages" with all complements. Such a conception is really too simple, and besides it takes no consideration of the facts. It is based upon a false idea, namely, that biological reactions are of the molecular order. An albumin functions as an antigen only while it maintains a colloidal state, it loses all interest from the biological point of view the moment that it passes, by virtue of a rupture in the colloidal equilibrium, to the state of a simple chemical substance.

Bordet sees in the fixation of complement "an act of adsorption comparable to the deposition of a dyestuff upon a solid object plunged into a bath, or to the fixation of ferments or toxins by precipitates or diverse pulverized materials such as animal charcoal, or better yet, to the phenomenon of mutual aggregation of the particles of two colloids when they are mixed." This conception certainly approaches most closely to the truth.

Needless to say, immediately after the discovery of this phenomenon it was seen to be a cause of immunity. The granular transformation became a "bacteriolysis;" the serum containing the sensitizer became a "bacteriolytic" serum. The experiments which follow will show that this interpretation is absolutely incorrect.

In the first place it should be stated that it is necessary to take certain precautions if we would be successful in this experiment. The culture of vibrios must be young, not more than six to eight hours old, for the vibrios with ageing normally assume the granular

form, although this does not interfere with their viability. Hueppe had noted these granules in old cultures a long time previously and had considered them as resistant forms, a view which was certainly correct.

Are the vibrios which are transformed into granules killed? Not at all. If one inoculates these altered vibrios into a culture medium they yield a growth of normal cholera organisms presenting their ordinary form of curved rods.

Does not the granular transformation render, at least, the organisms more fragile? Quite the contrary, as the following experiment shows. Many bacteria are quickly killed when suspended in salt solution if they are maintained at a temperature of 37 to 38°C., that is, at body temperature. The cholera vibrio, among others, will not resist under these conditions the destructive action of the saline for more than twenty-four to forty-eight hours. But Leuchs[1] has shown, and quite independently I have confirmed his observation, that it is only necessary to add to the saline a quantity, either large or small, of fresh serum taken from an animal prepared by injections of the cholera vibrio, and consequently capable of bringing about the transformation of the vibrios, to prolong for a considerable time the life of the organisms. While in the control tubes, containing a suspension of the cholera vibrios in saline all of the vibrios have been killed after forty-eight hours, they are still viable after eight days in a similar suspension containing in addition the fresh serum. The granular form, then, constitutes a form of resistance, a form which, moreover, appears normally in cultures. Furthermore, this granular transformation is an artificial phenomenon which does not normally take place in the body, and it is to Bordet himself that we owe the proof of this. He injected into the jugular vein of a rabbit well prepared by several injections of the cholera vibrio, a suspension of these bacteria. After an half hour he killed the rabbit and demonstrated that the vibrios had maintained their normal form and that they were still alive.

The explanation of these contradictory results is based upon the fact that there is no free complement in the circulating blood. This seemed to be true from a number of experiments, and Woll-

[1] Arch. f. Hyg., 1905, **54**, 396.

mann has provided the final proof. He exposed the jugular vein
of a rabbit, applied two ligatures, and injected into the tied off
segment of the vein a suspension of cholera vibrios previously
treated with sensitizer. As we have seen above, such vibrios are
transformed into granules in vitro within the space of three or
four minutes after they are placed in a fresh serum. But, in the
experiment of Wollmann no such transformation occurred, even
after a quarter of an hour. The conclusion appears unavoidable
that the conditions necessary for the phenomenon to take place
are not realized *naturally* in the animal; they obtain only *experi-
mentally* in the peritoneum or in vitro.

There is, moreover, a fact which seems of itself to be sufficient
to destroy the hypothesis that the serum of a prepared animal
enjoys "bacteriolytic" properties, and this is that the phenomenon
of transformation is observed with the vibrios only. Numerous
investigators have tried to reproduce this phenomenon with many
other bacteria, with results which have always been negative.
"Bacteriolytic" power is reduced, then, to a granular transforma-
tion of a single type of vibrio, and this granular transformation,
which has nothing in common with a "lysis," modifies neither the
vitality nor the properties of the organisms.

THE DIFFUSION OF HEMOGLOBIN

The phenomenon of complement fixation is manifested further
in the case of the red blood cells.

Let us prepare, as did Ehrlich and Morgenroth, a guinea-pig
by a series of three or four subcutaneous or intraperitoneal injec-
tions of red blood cells from the rabbit, given at eight-day inter-
vals. Ten days after the last injection bleed the pig, allow the
blood to coagulate, and remove the serum which exudes from the
clot. This serum contains, as we have seen, a specific precipitin
for the red cells of the rabbit. If we make a suspension of these
cells in physiological saline it is possible to provoke their agglu-
tination in large masses by the addition of this serum. But this
phenomenon is followed by a second. One sees, little by little,
the saline assume a red tint, becoming more and more pronounced.
This phenomenon has been christened by the name "hemolysis."
This word does not seem to be well chosen for it indicates that

under the influence of the serum there occurs a fusion of the erythrocytes, for the term "lysis" comes from the Greek word λύσις which signifies a dissolution. It can be shown that, in reality, the stroma, the skeleton of the red cell, is not touched, it remains intact; there is simply a *diffusion of the hemoglobin* into the medium.

Red blood cells are not living cells in the strict sense of the word, for they are not able to reproduce. Among the vertebrates the red cells are only the fragments of cells, particles of protoplasm impregnated with hemoglobin. Differing from those cells which are not attacked by the digestive ferments before having been "killed" by coagulation, the red cells are profoundly altered.

In the blood there is an isotonicity between the red cell and the blood plasma, that is to say, the osmotic pressure of the interior of the red cell is the same as that of the medium with which it is bathed. If we introduce erythrocytes into pure water the hemoglobin diffuses immediately as a result of the internal tension which is not counterbalanced. But the stroma subsists, although decolorized. If we place the red cells into water containing a quantity of salt adequate to render the osmotic pressure of the solution equal to that of the blood plasma, that is, in 0.8 per cent saline, the erythrocytes remain intact for a very long time. If we prepare several tubes containing water with decreasing amounts of salt, 5, 4, and 3 parts per thousand, and if we introduce the cells into these solutions we will see a slow diffusion of hemoglobin in the first, more rapid in the second, and so on, being the more active and the more complete as the quantity of salt, and of necessity the osmotic pressure, is less. The stability of the red cell results then from an isotonicity between the contents of the cell itself and the medium in which it is immersed. But still other conditions are essential to preserve this stability.

A red cell is composed of a skeleton, the stroma, formed of an albuminoid gel. This gel is impregnated with hemoglobin which, also, is in a colloidal state.[2] In view of what we know of the structure of gels it must be assumed that the micellae of hemoglobin are

[2] Certain authors have a very simple idea of the structure of a red cell; they represent it as being composed of a minute sac filled with hemoglobin.

retained in the network, extremely tenuous, of the albuminous gel. And it is clear that the diffusion of the micellae of hemoglobin may be brought about by very different means.

There can not be an hemolysis, or rather an hematolysis, except by a dissemination of the micellae of the gel, and this would result in the actual breaking down of the cell. But the contrary action, coagulation, ought to provoke a simple diffusion of the hemoglobin as a result of the dislocation of the stroma.

Corrosive sublimate coagulates albuminoid materials energetically. If we introduce red cells into a concentrated solution of this salt there is a coagulation en masse, both of the colloidal hemoglobin and of the colloidal albumin of the gel. Diffusion does not take place. But examination of the stroma which remains, after the diffusion of the hemoglobin is brought about by the serum of a prepared animal, shows clearly that here there is likewise a coagulation of the stroma. In effect, whether the diffusion takes place under the action of a substance which is able to incite nothing but a coagulation of the albumin, such as an acid or corrosive sublimate, or whether it be produced under the influence of a serum, the aspect of the stroma of the cell is the same. It has become rigid. Immersed in water with a high percentage of salt it does not contract, plunged into distilled water it does not expand. But if the diffusion is produced by a non-coagulating fluid, such as distilled water, it retains, on the contrary, its properties of an osmotic membrane.

We see further that the fixation of complement upon the antigen-sensitizer complex appears to be accompanied by the liberation of positive ions. In the case of the diffusion of hemoglobin under the influence of the serum of a prepared animal the positive ions must be provided by a metallic salt. In fact, the diffusion only takes place in a medium containing an electrolyte, which is normally sodium chloride.

It should be recalled that the presence of an electrolyte is also essential for the production of the flocculation reaction.

In brief, the final result of the antigen-antibody reaction, whether it reveals itself as a precipitation, as an agglutination, or as a fixation of complement, is always a flocculation.

THE FIXATION OF COMPLEMENT

Let us consider further the peculiarities involved in the diffusion of hemoglobin as effected by the serum of a prepared animal. All of these peculiarities are identical with those presented in the phenomenon of the granular transformation of vibrios. The diffusion of hemoglobin is brought about by the action of two principles. The one, the complement, is destroyed by heating at 55°C., and is found in the serum of all animals, and experiment shows that it is the same, whether it operates on red cells or upon vibrios. It only provokes the diffusion of hemoglobin if the cell has been previously reacted upon by sensitizer. The second factor, the sensitizer, is specific. It is present in appreciable quantity only in the blood of prepared animals. It is only fixed to the red cells of individuals appertaining to a species of animal identical with, or very closely allied to, that furnishing the cells which were introduced into the body of the animal yielding the serum.

As we have seen with reference to the precipitins, the formation of a specific sensitizer is also produced only if there exists a difference in physical state between the albuminous colloids injected and the corresponding albuminous colloids of the body of the animal which receives the injection. In so far as sensitizers are concerned, this difference in physical state may be extremely small, for it may be observed that one is able to obtain the formation of a specific sensitizer if one injects an animal with the red cells of another animal of the same species, but of a different race.

A guinea-pig is prepared by injections of the cells of the rabbit. Its fresh serum attacks violently the cells of the rabbit, weakly those of the rat and the mouse, and not at all those of other animals. A rabbit prepared with horse red cells causes the diffusion of hemoglobin from the cells of the horse and the donkey only. We meet here again a relative specificity such as we found in the flocculation of sera by the precipitins. The two actions can always be superimposed one upon the other, with the restriction, however, that the precipitins show a somewhat more strict specificity. Precipitins and amboceptors allow us to settle questions of parentage among the different animal species, or, on the contrary to establish their lack of relationship.

We have seen above that the sensitizer is found in appreciable quantity only in prepared animals. We may observe, in fact, that even the serum of a normal individual may cause the diffusion of the hemoglobin from the cells taken from other individuals pertaining to certain species of animals. It is thus, for example, that human serum normally attacks the cells of the sheep. But this action of normal serum is in no case comparable in intensity to that of the sera of prepared animals.

The sensitizer is firmly retained by the cells to which it is fixed and can not be removed by repeated washing. On the other hand, if one immerses a sufficient quantity of red cells modified by the specific sensitizer into a fresh serum the complement contained in the latter disappears; it is adsorbed by the sensitized cells. All of these reactions take place in a manner analogous to those involved in the granular transformation of vibrios.

THE GENERAL NATURE OF THE PHENOMENON

From these facts Bordet and Gengou have demonstrated that the formation of specific sensitizer is a general phenomenon, taking place in the blood of animals prepared by the injection, or better by a series of injections, of any colloidal albumin whatever which can serve as an antigen. But it is only in the case of the cholera vibrio and of the red blood cell that the reaction can be demonstrated directly.

Let us prepare a rabbit by the administration of typhoid bacilli, giving the animal a series of three or four injections. A precipitin and a specific sensitizer will appear simultaneously. The presence of the precipitin can be very readily verified, that of the sensitizer can only be disclosed indirectly for the bacilli placed in contact with this serum do not manifest to any degree a granular transformation nor are they modified either as to their form or properties. Nevertheless the serum contains specific sensitizer, as can be demonstrated by the following method devised by Bordet and Gengou.

Place in a test tube 2 cc. of a suspension of typhoid bacilli in physiological saline. Add to this suspension a few drops of *fresh* serum from a prepared rabbit. This serum contains complement certainly, since it is present in the blood of all animals. In addi-

tion it contains sensitizer, or at least something which impregnates the bodies of the typhoid bacilli and which allows complement to be fixed to them, since the complement disappears from the mixture.

As a second part of the experiment let us introduce sheep cells into the serum (previously heated to 55°C. to destroy the complement) of a rabbit prepared by injections of sheep cells. These cells become impregnated with sensitizer. If we place such cells in a liquid containing complement the diffusion of hemoglobin is brought about; if complement is not present diffusion does not take place.

Add, then, these cells to the suspension of typhoid bacilli which have been in contact with the serum in which it was desired to demonstrate the presence of an elective sensitizer for these bacilli. If it is found that the cells remain intact the liquid must have been deprived of the complement which it contained. This complement must therefore have been fixed to the bacilli, but for this it was requisite that the bacilli be made receptive, which could only be the case if they were impregnated with sensitizer. It must be concluded that a specific sensitizer existed then in the serum of the rabbit prepared by injections of this bacillus.

Repeat the same experiment, using the same rabbit anti-typhoid serum, but combining it with any bacterium other than *B. typhosus*. The red cells which have been impregnated with sensitizer and then added to the serum-bacterium mixture undergo a diffusion of the hemoglobin, for the sensitizer, which is specific for the albumins present in typhoid bacilli, does not unite with the albumins of a bacterium of another species. The complement, unable to bind itself to a sensitized albumin, remains in solution and is ready to act upon the previously sensitized red cells of the sheep.

In this way it can be proved that each time that there occurs within the organism an invasion, either experimental or natural, of a bacterium there is the production of a sensitizer specific for this bacterium. In a word, all bacterial albumins function as antigens, but the intensity of sensitizer production is markedly variable, differing from one bacterium to another, and differing likewise, for the same bacterium, from one animal species to

another. This fact is expressed by saying that certain bacteria are good antigens, others poor; that certain animals are good antibody producers, others poor producers of antibody. What is concealed within all these words? This is what we must attempt to discover.

As a general rule, all bacteria which provoke the formation of a potent precipitin provoke also the formation of an active sensitizer, and inversely. Probably but a single phenomenon is involved.

The reaction of Bordet-Gengou, called the complement fixation reaction, can be used to establish the identity of a given bacterium of which the nature is doubtful, or it can be applied to the diagnosis of certain human or animal diseases.

In the first case, the test consists simply in determining if the bacterium in question fixes complement in the presence of a serum containing a specific sensitizer developed by the injection of bacteria of a known type. For example, if it is necessary to confirm the nature of an organism which presents the characteristics of a dysentery bacillus it is only necessary to see if this bacillus fixes the complement in the presence of serum from an animal prepared by injections of an authentic dysentery strain.

In the second case, where it is desired to confirm a clinical diagnosis of a disease, blood is removed from the patient and the serum is examined for a sensitizer specific for the bacterial agent of the disease with which the patient is supposed to be infected.

Technically, the specific precipitin test is more readily performed than is that of fixation and except in special cases, is the one usually employed. In the case of the tubercle bacillus, for example, it is difficult for technical reasons, to detect the presence of antibody in the blood of patients by agglutination, but on the contrary, it can be readily demonstrated by fixation.

THE WASSERMANN REACTION

It may be wise to consider for a moment a reaction which has been derived from that of Bordet and Gengou and which, at first sight, presents a certain analogy to the latter, being based upon the phenomenon of complement fixation. This is the so-called Wassermann reaction, used in the diagnosis of syphilitic infection. It is of particular interest in that, although it shows very well the

fixation of complement, it is the result of a disturbed colloidal equilibrium.

At the time of the discovery of the reaction the organism of syphilis, *Treponema pallidum*, had not been successfully cultivated in vitro. Thus, Wassermann sought to surmount the difficulty of diagnosis by employing a fixation reaction. This he did as follows.

An aqueous extract was prepared of the liver of a stillborn syphilitic infant. This tissue was selected since it had been shown that such a liver contained an abundance of the spirochetes. From the patient, that is, the individual supposedly affected with syphilis, blood was taken and the serum was heated to 55°C. to destroy the native complement.[3] Into a test-tube containing 1 cc. of physiological saline, 4 drops of the suspected serum, 4 drops of the extract of syphilitic liver, and 2 drops of guinea-pig serum were placed. The mixture was incubated in a water-bath at 37°C. for one hour to give the reaction time to be effected. What happened? If the serum in question was derived from a patient really syphilitic the sensitizer combined with the bacterial albumins, present in solution in the liver extract, and formed a complex which fixed the complement which was free in the mixture. If, on the contrary, the individual who furnished the serum was not syphilitic nothing at all happened. For a normal serum does not contain a specific sensitizer, hence the complement remained free and ready to unite with any suitable antigen-sensitizer complex. Thus, to have a positive or a negative reply to the question of syphilitic infection it was only necessary to determine if free complement remained in the solution. To ascertain this, 2 drops of a suspension of sheep cells in saline and 2 drops of a heated serum obtained from an animal prepared by injections of sheep cells were added. This last serum contains a sensitizer which would impregnate the red cells, and only complement would be lacking

[3] It has been found that it is possible to utilize the native complement in the blood of the patient, and this is what is done in several simplified procedures. But as the complement deteriorates rapidly, and because the complement content of different bloods varies very greatly it is preferable to destroy this unknown and variable amount of complement and to use as complement the serum of normal guinea-pigs which can be exactly titrated.

to bring about hemolysis. Therefore, if diffusion of hemoglobin did not take place it was because there was no complement free; it had been fixed in the first part of the reaction and this proved that the serum contained a sensitizer and was derived from a patient with syphilis. If, on the contrary, the diffusion of hemoglobin did not take place, it was because nothing transpired in the first part of the reaction, no sensitizer was present, and the serum was derived from a person without syphilitic infection.

Up to this point, indeed, all is in beautiful accord with the theory of the specific fixation of complement. But one must not hesitate to recognize that the reaction will proceed in exactly the same way, giving the same results, if the extract of liver, the antigen, is made from a normal liver, completely free of syphilis. And it will be further seen that in the place of human liver, any organ taken from any animal whatever—extract of beef heart, of calf, or of guinea-pig heart—serves perfectly. This is somewhat disconcerting, for this extract of heart most assuredly contains no albumins derived from the spirochete of syphilis. It can not be a question of the fixation of complement through a specific sensitizer. What, then, brings about this reaction, and why is it effected only with the serum of syphilitics? It occurs because only in syphilis is there a profound disturbance in the equilibrium of the serum colloids. This instability of syphilitic sera is demonstrated by the facility with which such a serum flocculates through the action of any colloid whatsoever.

In all cases an analysis of the phenomenon leads to the same conclusions. There is a flocculation under the influence of a specific precipitin, there is in fixation the formation of a complex which absorbs complement. Essentially these reactions depend upon a coagulation; they are always phenomena where the equilibrium of the colloids plays a most important rôle.

THE EFFECT OF FIXATION UPON BACTERIA

We have seen above that bacteria placed in a mixture of specific sensitizer and complement are not modified at all, and that one must resort to an experimental artifice to show that they have actually absorbed the sensitizer. The vitality of these bacteria is not affected, their properties are not interfered with, and the

experiment cited in connection with the vibrios permits us to show that it is the same with all types of bacteria. All remain alive much longer in physiological saline to which has been added a fresh specific serum (necessarily containing the sensitizer specific for the bacterium and complement) than they do in saline alone.

Why do they not undergo a phenomenon analogous to that of the diffusion of hemoglobin? Simply because there is no resemblance between a red blood cell, an *albuminous gel* impregnated with hemoglobin, and a bacterium, formed almost entirely of nucleoproteins, a living cell in all that that term connotes.

That which leads to the appearance of fixative power is in all cases the colloidal albumin. This fixative power appears not only upon the injection of the intact red cell but also if only the stroma of the cell is used, although it may be crushed or modified in any way whatsoever, provided that its colloidal state is respected. It is known that the red cells of birds and of reptiles have a nucleus. With these cells only the protoplasmic albumin is antigenic, the nucleus is not. If one causes the fresh serum of an animal prepared by injections of the cells of one of these animals, a pigeon for example, to act upon the cells of the pigeon, there is a diffusion of the hemoglobin, but the nucleus is not touched. The nucleus is formed of nucleoproteins, substances which have no antigenic function, and which do not provoke the formation of antibody, either precipitin or sensitizer.

But of what is a microörganism, a bacterium, composed? Almost entirely of nucleoproteins. The quantity of albumin in a bacterium is extremely small. Antibodies exercise no action upon the essential mass of this bacterium.

The specific sensitizer appears in an animal into which is injected only the albumin isolated from the bacterial body. It is likewise the albumin alone, whether incorporated in the bacterial body or free in the liquid which forms with the specific sensitizer a complex to which the complement adheres.

Complement provokes a coagulation of the albumin which comprises the essential portion of the stroma of the red cell, those fragments of protoplasm which submit passively to the forces of the environment, and that which causes a disorganization of the red cell brings about a diffusion of the hemoglobin. Is this coagula-

tion not produced even on the minimal quantities of albumin contained within the bacterium, which, because living, does not submit passively to the influences of the environment? Most probably not, since its vitality is in no way affected.

This difference of reaction between the red cells on one hand and the bacteria on the other is evidenced with respect to their susceptibility to the proteolytic ferments. Ferments exercise no action upon living bacteria; perfectly normal cultures of bacteria may develop in media containing large amounts of ferment. On the contrary, the erythrocytes are profoundly altered if they are placed under such conditions.

And finally, within the body bacteria do not fix complement, as several investigators, particularly Wollmann, have conclusively shown, and for the reason that either there is no complement in the *circulating* blood or this complement will not act under the experimental conditions. It is quite essential to remember always that we are dealing with colloidal reactions which depend upon the state of equilibrium of the colloids present, and it is very certain that the equilibrium of the colloids of the circulating blood is far from being the same as that of the blood outside of the body, in the test-tube. It is by no means certain that these antibodies—precipitins, sensitizers—exist as such, as chemical substances. They may represent a particular state of the colloids of the blood, and the same observation applies, with much more reason, to complement.

There are, moreover, other facts which show that the result of the fixation of complement reaction is very different in vivo from what it is in vitro, even in the case of elements whose integrity may be affected. The formation of sensitizers to diverse cells of the body has been attempted and experiment has shown that such reacting bodies can be produced. Among the first to attempt this was Metchnikoff, who obtained a serum containing a sensitizer specific for the white blood cells by injecting an animal with the leucocytic exudate from an animal of another species. In vitro leucocytes which have fixed complement do not show any profound changes, and this fixation can only be shown to have taken place by means of the experiment of Bordet already cited. As Metchnikoff attributed solely to the leucocytes the defense of the body

against bacteria he hoped that it would suffice to inject an animal with a serum containing such a specific sensitizer to render this animal more sensitive to infection. Experiment did not give him the expected result, and for a simple reason—the leucocytes were not modified.

But most characteristic from this point of view is the serum which the partisans of humoral immunity have termed "spermotoxic." Such a serum is prepared by injecting an animal with the spermatozoa of an individual of another species. (In this specific case it is even possible to obtain the formation of a specific sensitizer by injecting the animal with his own spermatozoa.) In vitro this serum manifests its action by immobilizing spermatozoa for which the sensitizer is specific. Some authors affirm without any proof, that these spermatozoa are even killed. But that is of no consequence, this is the experiment always cited as demonstrating that the phenomenon is produced in the body in the same manner as in the test-tube. But indeed, the prepared guinea-pig, for example, whose blood contains specific sensitizer (since it has received injections of its own spermatozoa) and complement (since the blood of all guinea-pigs contains it), behaves but very poorly, for it may be shown that his spermatozoa are not immobilized, nor are they killed, nor do they undergo any change whatever, for this guinea-pig is as capable of fertilizing a female as is a normal guinea-pig.

Might it be said that this fact is explained as a lack of penetration of the sensitizer to the seminal glands? This would be absurd, a priori, and moreover it can be demonstrated that the spermatozoa are united with the sensitizer in the organ itself, for these spermatozoa placed in the presence of normal guinea-pig serum, containing complement only, fix complement.

All of these experiments agree, then, to demonstrate that the fixation of complement by an autonomous living cell, a bacterium, does not exert any action upon it, modifying in no way its vitality, its power of reproduction, nor any of its properties, whether the action takes place in vitro or in vivo. They show equally well that such fixation does not take place, normally, within the body. It is an artificial phenomenon. The reaction does not take place within the blood for a very fundamental reason, namely, that the

complement itself is an artificial principle, a product of the laboratory.

Recently Sachs and Morgenroth have shown that alexin is not a substance but that it is rather a state of equilibrium of the serum globulins. But these authors have not gone far enough and have not drawn the logical conclusion from this discovery, a deduction in accord with the experiments which show that the phenomenon of complement fixation does not normally take place in the body.

We know, beyond any possible doubt, that the state of equilibrium of the globulins of the circulating blood is not at all the state of equilibrium of these same globulins[4] in the plasma or in the serum which is expressed from the blood clot after coagulation. Since alexin is a state of instable equilibrium of the globulins of the serum, this state of equilibrium can not be that of the globulins of the circulating blood, a fact which explains the situation; the phenomenon of fixation does not take place within the circulating blood because the "alexic equilibrium" is lacking. This state of alexic equilibrium of the serum is, moreover, very instable disappearing within a few days, and this can be readily conceived since we know that the state of equilibrium of colloidal sols changes

[4] But in the first place, do the constituents of the plasma—fibrinogen, globulin, albumin—represent distinct substances or are they differing "colloidal states" of the same albuminous substance (an idea first presented by Herzfeld and Klinger of Zürich) which is poured into the blood from the liver, and in which the micellae stabilize themselves little by little under the influence of the different products circulating in the blood? That which renders this conception possible is that in reality there is not one fibrinogen, one globulin, one albumin (substances which we can distinguish from each other because of their greater or less stability toward precipitating reagents) but a continuous series of fibrinogens, of globulins, and of albumins, passing insensibly from the one to the others. The condition is only understood when we picture these "bodies" as "states of equilibrium," more and more stable, of the same colloidal sol.

Fibrinogen, which plays the principal rôle in the coagulation can not be, then, a chemical substance distinct from the other elements of the plasma, but a state of equilibrium, particularly unstable, of the single albuminous sol constituting the plasma. It must be the same for the other elements entering into the reaction. But in the present state of our knowledge it is impossible to define exactly these "states of equilibrium."

spontaneously in the direction of a flocculation.   If the serum is
heated to about 57°C. the alexic property is lost and at the same
time the equilibrium is modified.   In a word, in this conception of
the fixation reaction the alexin is a labile state of equilibrium of
fresh serum.[5]

UNICITY OF THE ANTIBODIES

We have seen that but a single phenomenon takes place under
the action of the antibodies—a flocculation.   Can, then, all of the
antibodies which have been described be reduced to a single one?
This hypothesis has been suggested by several scientists, but the
idea has not been generally accepted up to the present, obviously
because it has been believed that the phenomena provoked have
been multiple.

In 1902 Gengou noted that alexin was fixed when a precipitating
serum was added to the homologous antigen, but he assumed the
simultaneous presence of "albuminolysin," although no lysis
took place.   Friedberger, in 1908, advanced a hypothesis regard-
ing the identity of precipitating antibody and the anaphylactic
antibody, and Doerr and Russ showed that the sensitization in
passive anaphylaxis was proportionate to the precipitating power
of the injected serum.   But it remained for Zinsser to be the cham-
pion of the concept of the unicity of the antibodies.   His experi-
ments have led to the conclusion that "there was no need for
assuming that the antibodies which were involved in the fixation
of the alexin were essentially different from those that brought
about the precipitation."

But for all authors up to now "bacteriolytic" action or "hemo-
lytic" action could not be questioned.   According to the condi-
tions of the experiment the antibodies caused a "lysis" or a "floc-
culation."   Zinsser states "The resultant reaction which may be
observed with this sensitized antigen (agglutination, precipitation,
complement fixation, bactericidal phenomena, bacteriolysis, opson-
ization, or sensitizing effects in the anaphylactic sense) would be
determined, not by differences in the nature of the antibodies
with which the antigen had united, but rather by the physical

[5] Les défenses de l'organisme.   Flammarion, Paris, 1923.

state of the antigen itself, the nature of the coöperative substance (alexin, leucocytes, tissue cells) and by the environmental conditions under which the observations are made.''[6]

When once it is established, as I believe to have shown, that lytic action never manifests itself, that the antibodies always exercise a coagulation phenomena, everything is explained. There is but a single antibody which provokes always the same phenomenon, a coagulation.

The facts are very simple. The apparent complication has been the result of the failure of the theories to consider the facts.

ACTION OF THE ANTIBODIES IN VIVO

All of the phenomena described—"agglutination," "complement fixation"—are then artificial phenomena which take place only within the test-tube. Can it further be said that the production of antibody within the body does not lead to any in vivo phenomena? By no means. But it is not because of the presence of a foreign *cell* that antibody is formed, it is because of the presence of the *albumins* of this cell dissolved in the blood. The formation of antibody is the expression of a reaction tending to reëstablish the colloidal equilibrium of the serum albumins, which have been disturbed by the invasion of the body by an heterologous albuminous colloid. The result is that it is not the foreign cell which undergoes the reactional shock, but the body itself!

The term sensibilisatrice, to designate this antibody, is particularly appropriate, but not in the sense in which it has been used. That which the antibody sensitizes is the body.

Such a reaction may be considered as a process of defense against albuminous colloids *in solution*, in the sense that it results in coagulation of the foreign albumin, that is to say, in its destruction as a colloid capable of influencing the equilibrium of the blood colloids. The coagulum is next eliminated, either through phagocytosis by the leucocytes, or by digestion in situ by the defense ferments which form in the blood at the same time as the coagulating antibody. This preliminary flocculation is essential to the digestion process, for colloids are immune to the attack of a ferment until they have undergone a previous coagulation.

[6] J. Immunol., 1921, **6**, 289.

Although the result of antibody action is a coagulation which permits maintenance of the integrity of the blood plasma, unfortunately these processes may be disastrous for the organism in which they take place.

A first introduction of an antigen into the body causes, at the same time that it leads to the appearance of a principle which provokes the flocculation of this antigen, the sensitization of the animal, by virtue of the formation of an instable state of equilibrium of the blood colloids. The acquisition of the sensitizing power predicates the *anaphylactic state*, and this instable equilibrium is susceptible to rupture under the effect of a second introduction of the same antigen. This rupture is *anaphylactic shock*.

The Real Results of the Reaction Against Colloids: Anaphylaxis

EXPERIMENTAL SHOCK

The sensitivity which animals manifest to a reinoculation of certain substances has been known for a long time. Magendie had observed in 1839 that rabbits which had received two intravenous injections of egg white might succumb suddenly as the result of a third. Flexner, and also Pfeiffer, had noted analogous facts in 1894, the first as the result of reinjections of serum, the second after repeated injections of the cholera vibrio. But these observations did not attract attention. In 1902 Portier and Richet, studying the action of the poison of the actinia[1] noted that dogs which had supported without inconvenience an intravenous injection of a small dose of an aqueous extract of the tentacles died regularly if they were injected by the same route, some days later, with a second dose, very small, and incapable of killing normal dogs. They termed this peculiar sensitivity anaphylaxis (that is, contra-protection). They considered, in fact, that instead of immunizing against the poison of the actinia, actinocongestin, the first injection had, on the contrary, sensitized the animal. In reality, and Richet himself recognized this a little later, the actinocongestin did not act in this respect as a poison but rather as an albuminous substance.

The work of Richet and Portier was the first of a series of elaborate investigations effected by a multitude of scientists. The singularity of the facts observed led to the emission of the most varied hypotheses concerning the intimate nature of the phenomena of sensitization.

Let us see, first, how these phenomena manifest themselves in the guinea-pig, the reagent of choice for the study of anaphy-

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[1] Marine polyps attached to rocks. *Actinia equina*, purple or green, is abundant on the coasts of France.

laxis. Inject a guinea-pig, subcutaneously, with a colloidal solution of any albumin, animal or vegetable in origin. Some days later give a new injection, by the intravenous route this time, of a small quantity of the same albumin. A few seconds later the guinea-pig presents a violent crisis, of short duration, which terminates usually in death; or, the animal recovers rapidly and within a few minutes regains its normal state.

Let us analyse this phenomenon. The first injection, called the sensitizing injection, may be extremely minute; as little as 0.001 mgm. of a purified vegetable albumin, of egg albumin, or of serum suffices. We find here the same conditions as those essential to the formation of antibodies. It is necessary that the albumin injected be heterologous with reference to the albumins of the animal which receives the injections. Likewise, only the colloidal albumins are capable of sensitizing, to the exclusion of all other colloids. In a word, any substance capable of functioning as an antigen, and such substances only, can sensitize an animal.

The sensitization is not produced immediately after the sensitizing injection. It is not established until after a period of incubation varying with the dose of albumin, being shorter as the dose is smaller. It never appears before the sixth day, and once established, it persists for a very long time, without doubt throughout life.

The second injection, called the intoxicating injection, must be relatively larger than the sensitizing injection. For a serum, for example, it should be at least a tenth of a cubic centimeter. The larger it is the more violent is the shock. The intoxicating injection, especially if the amount is small, should be given quickly at a single time, and it is expedient to adopt by preference the intravenous route.

THE PECULIARITIES OF SHOCK

The sensitizing and intoxicating injections must be effected with the same albumin. If one sensitizes with egg white the crisis appears only as a result of the reinjection of egg white; if the sensitization has been produced with horse serum only the reinjection of horse serum is able to induce the crisis, to the exclusion of all other sera.

It can be seen that in anaphylaxis we have, again, a method for determining the parentage of animal species, their closeness or remoteness, and the results as disclosed by anaphylaxis are comparable to those given by the antibody reactions. It could not be otherwise, since the anaphylactic state is determined by the antibodies.

The anaphylactic reaction allows us to solve certain problems of legal medicine and of hygiene; to establish the nature of a blood or of an animal product for example. If one would prove that a trace of blood is human blood it is only necessary to sensitize a guinea-pig with some of the dissolved stain, and as a thousandth of a milligram of serum suffices to sensitize, a very small stain will yield an adequate amount of serum. Twelve days after the injection of the suspected blood reinject the pig intravenously with a cubic centimeter of known human blood. If the crisis is produced, it follows that the pig was sensitized to human blood, proving that the suspicious stain was due to blood of human origin, with this single proviso, it may have been the blood of a higher monkey, for in this reaction again it is impossible to establish a difference. The relationship is too close.

It is even possible, as has been shown by d'Herelle and Géry, to differentiate the blood of the female from that of the male. Thus, a guinea-pig which has received a sensitizing injection of a minute amount of an extract of placenta will react to the serum derived from the female, whatever may be the age of the female— fetus or a very aged woman—and it will not be at all sensitive to the serum of males, even if the serum is taken from a male fetus.

THE SYMPTOMS OF SHOCK

When the intoxicating injection is very large, 4 or 5 cc., in the case of serum, the animal succumbs very suddenly, within a few seconds. More interesting is the shock induced by a smaller quantity, such as 0.25 to 0.5 cc. One or two minutes after the inoculation the guinea-pig becomes restless, suddenly shakes himself, scratches and coughs. Very quickly the movements become disordered, then convulsive, and at the same time an intense dyspnea appears. There is emission of urine and feces. The animal falls upon its side, paws with its feet and appears

to suffocate. Then prostration occurs, the respirations become less and less frequent, the uncoördinated movements cease and the animal succumbs. The whole reaction does not last more than a few minutes.

At times, however, the crisis terminates in a wholly different manner. All of the symptoms are the same, the animal falls prostrate and then, within the space of a few minutes, the respiration returns to normal and the guinea-pig is restored to a condition of perfect health. The crisis has left no trace.

Shock is accompanied by a fall in temperature in cases which are fatal, by an hyperthermia in those which recover.

The smaller the intoxicating dose the higher the percentage of sensitized animals which survive. The crisis is also less and less severe, and with doses very small the animal shows only restlessness, scratching and coughing, with a complete return to normal within a very few minutes.

Injection by the intravenous route is not the only method which may lead to the anaphylactic crisis, but as the initial cause of the crisis is the penetration of the foreign albumin into the circulation the dose introduced into the body by any other avenue must be higher since the passage of the albumin into the circulation will be slower. The quantity must therefore be somewhat greater if the intrapleural route is used, a little greater still if it is administered intraperitoneally, much greater if it is given subcutaneously, and finally, massive if the albumin is ingested. As to this last method, we have already seen that in the case of the ingestion of a considerable amount of albumin the digestive processes may be overwhelmed and a certain quantity may, in its native form, penetrate the walls of the digestive tube. Nevertheless, although certain anaphylactic disturbances may be observed as the result of the ingestion of albumin by an animal sensitized to this albumin, the disturbances are in all cases relatively slight and the result is never fatal.

THE ANALOGY BETWEEN ANAPHYLACTIC SHOCK AND PROTEIN SHOCK

For a long time it has been known that a shock presenting all of the characteristics of the anaphylactic crisis could be produced

in the most varied animals by a single injection of any albuminous substance. Délezenne has indicated that the injection of all albumins produces identical reactions in the blood. Arthus finally showed that this similarity of action resulted naturally from a similarity of cause; it is not the peptone, the serum albumin, the ovalbumin, or even the venom of the serpent which acts, as a chemical substance, they all act rather as colloidal substances.

We will see in a moment that it is possible to desensitize a sensitized animal, and this, simply by fractioning the intoxicating dose. Physiologists had known for a long time that protein shock could be prevented by injecting a short time before the massive injection a minimal amount of the harmful substance. That which is peculiar to anaphylaxis is that the first injection creates a special sensitivity, such that a second injection of a minimal quantity suffices to provoke a crisis, which, without the preliminary sensitization, would have required a massive single injection. Nevertheless, in the two cases, the intimate causes of the crises are identical; there is a sudden rupture in the colloidal equilibrium of the blood, revealed, as has been shown by Widal, by a sudden variation in the refractometric index of the serum.

THE ANAPHYLACTIC CRISIS IN PATHOLOGY

The anaphylactic crisis has assumed a great importance in human pathology, as a result, especially, of the work of Widal and his students. In man, the symptoms are similar to those presented by animals—dyspnea, convulsive phenomena, gastro-intestinal disturbances, collapse—unfolding rapidly and always preceded by arterial hypotension.

Sensitization may be produced naturally by the penetration into the circulation of native albumins, either by passage through the digestive mucosa, or by penetration through the mucosa of the respiratory tract.

In man, alimentary disturbances are often observed presenting symptoms which warrant the conclusion that such intolerances are of anaphylactic nature. It is necessary to remark, on this subject, that these accidents never present exceptional severity as in the crisis which follows an intravenous intoxicating injection. There is not, properly speaking, an abrupt crisis but an intolerance,

such as can be readily produced in the animal.   Inject a rabbit,
as Arthus has done, by the subcutaneous route with 5 cc. of
horse serum.   No disturbance results and the injected material
is absorbed rapidly.   Give a second injection, equal to the first,
eight days later, and likewise subcutaneously.   Resorption
takes place as formerly.   Continue to give weekly injections of
5 cc. of horse serum.   The fluid of the third injection is absorbed
more slowly than that of the second, the material of the fourth
still more slowly, and an exudation, followed by a scab, may
be observed at the site of the injection.   The fifth, and subsequent
injections, always give these local disturbances.   Gradually
the rabbit emaciates and at length dies.   Such anaphylactic
phenomena may be obtained with any albumin whatever.   If the
rabbit is sensitized only to horse serum we may substitute for
the weekly injection of horse serum an injection of sheep serum,
even at a time when the local reactions are most severe, and
the fluid will be absorbed perfectly.   The intolerance is therefore
specific.

The anaphylactic reaction assumes different forms according to
the mode of re-introduction of the albumin.   The anaphylactic
crisis results from the sudden appearance of an heterologous
albumin in the circulation of a sensitized animal.   Anaphylactic
intolerance is chiefly marked by a local disturbance.   But since,
on the other hand, a certain quantity of the albumin injected
into the tissues penetrates slowly into the circulation, intolerance
may be accompanied by general disturbances more or less severe
according to the rapidity of the transfer.   It may be noted that in
the rabbit described above the resorption of injected fluid took
place more and more slowly.   This may be considered as a local
reaction of defense, tending to inhibit the passage of albumin
into the circulation and consequently opposing the anaphylactic
crisis.

The phenomena of alimentary intolerance in man manifest
themselves in many individuals, and in certain cases it is possible
to ascertain the origin of the sensitization.   Intolerance to eggs,
to crustacea, to the flesh, and especially the milt of fish, or to
strawberries is common.   It often has its origin, either in an
excessive consumption (this is particularly true of intolerance to

eggs), or through the absorption of a spoiled product. In this last case the passage of traces of native albumin through the mucosa is favored by a digestive disturbance.

The most common symptom of alimentary intolerance is urticaria, such as we see as a characteristic symptom in serum disease. Then come the intestinal disturbances. The cause of the last is most probably an inflammation of the mucosa resulting from a reaction of defense of the same nature as the local cutaneous lesion of the rabbit. As for the urticaria, it represents a true anaphylactic crisis.

Widal has followed a patient who presented an intense urticaria each time that he absorbed an animal albumin, although no disturbance took place while on a vegetable diet. The sensitization was effected by the consumption of spoiled meat. About one hour after a meal composed of animal albumin there occurred a characteristic fall in blood pressure, the hemoclastic crisis, which developed without outward symptoms, and it was only later, when this crisis was ended and the pressure had returned to its normal level, that he showed the urticarial wheals. Cordier has demonstrated the same facts on other subjects affected by intolerance manifesting itself by intestinal symptoms.

Foreign albumins may gain entrance to the circulation not only by way of the digestive tract, but also, and even more readily, by the pulmonary route. Hay fever and certain types of asthma are certainly of anaphylactic origin.

There is still another cause, and a very important one, which may lead to anaphylactic accidents. The bodies of bacteria contain a very small amount of albumin. Among the diverse symptoms which characterize a given infectious disease the greater part are specific, that is to say, they pertain to this disease solely and they are related to the action of products elaborated by the bacteria with which we are not here concerned. But other symptoms are common to all infectious diseases, they have a common cause, and are due to the protein nature of the bacterial substance. It is necessary to distinguish, then, in each disease the part of the symptom-complex resulting from the bacteria by virtue of their toxic substances and that part which pertains to the colloidal substances of the bacteria. We will return to this subject later.

Not only in a natural manner in the course of disease do bacterial colloids gain entrance to the body. They gain access, and in a manner still more abrupt, in the course of antityphoid or anticholera vaccination, in fact, every time that bacterial albumins are injected. There exists here an additional aggravating circumstance, which is that the majority of the methods of vaccination provide for several injections with an interval of eight to ten days between each of them. Thus are realized the best conditions for the production of an anaphylactic crisis. Since such vaccinations are usually carried out with bacterial bodies which have been heated the danger is not, indeed, great, and is the less the higher the temperature used to kill the bacteria, the colloidal state of the albumins being the more altered the higher the temperature to which they have been subjected. In fact, serious accidents never result from the injection of *heated* vaccines, but the danger becomes considerable when a series of injections is given with a colloidal solution of unheated bacterial proteins. In this case the first injection may cause protein shock, and reinjections may lead to a serious, even fatal, anaphylactic crisis. The reaction to vaccine may assume one of two forms. It may be characterized by disturbances which do not become severe, such as fever, headache, lameness, and lack of appetite, symptoms which are due to the bacterial toxins; or it may induce vaccine shock, characterized by dyspnea, the tendency to syncope, cyanosis, and anuria. The latter is purely a protein, or anaphylactic reaction and is observed only following reinjections of bacteria in colloidal solution.

THE LESIONS OF SHOCK

Autopsy of a guinea-pig dead of anaphylactic shock reveals an intense congestion of the digestive tract and of the lungs; the tissues of these organs are frequently studded with hemorrhagic foci. The lung is distended, filled with air; the tissue is rigid and no longer collapses. The blood coagulates but very slowly.

It should be observed that the symptoms and the organic lesions of anaphylactic shock are absolutely identical whatever may have been the albumin which led to their appearance, whether it be an albumin lacking all toxic properties, or a poisonous albumin as is, for example, actinocongestin, crepitin, or even cobra venom, or

yet an albumin derived from bacterial cells. For these last the toxicity plays no part whatever in the appearance of the anaphylactic crisis.

The anaphylactic crisis begins with a constant phenomenon; prior to all outward symptoms there is a sudden fall in arterial pressure. Whatever may be the species of animal, whatever may be the severity and the outcome of the shock, the abrupt fall in arterial pressure always takes place, but it is, however, much more marked when the shock is severe.

This finding seems to show that the anaphylactic crisis may well be a result of a sudden rupture of the colloidal equilibrium of the blood. We will see that other facts confirm this hypothesis. For the moment we will be content to state that the possibility of desensitizing an anaphylactic animal accords perfectly with this point of view.

DESENSITIZATION

Take a guinea-pig sensitized to horse serum. Twelve to fifteen days after the sensitization inject intravenously a very minute amount of horse serum, a quantity by itself incapable of causing any disturbance, 0.001 cc. for example. Then a few minutes later inject a slightly larger dose, 0.01 cc., and after another short interval 0.05 cc., then 0.1 cc. At this time this last dose will not cause any trouble although if it had been injected at first it would certainly have brought on a severe, if not a fatal shock. After a few minutes more it is possible to give the pig an intravenous injection of a large dose of serum, several cubic centimeters. This quantity given without preparation would have been immediately fatal. Instead of giving a series of small, gradually increasing injections it is possible to prevent the anaphylactic crisis by simply taking care that the serum injected intravenously be administered with extreme slowness. This method of desensitization, by "sub-intrantes" injections, discovered by Besredka, is general. It is applicable to all animals. But an important point should be noted—this desensitization is only transitory.

SERUM DISEASE

The guinea-pig, as we have said, is the reagent of choice for the study of anaphylaxis. It is in this animal that the crisis reveals its character in the most dramatic manner, but it is nevertheless a general phenomenon. All animals are sensitized by a first introduction of antigen into the circulation and are liable to shock upon a second introduction of the same antigen. It appears from the experiments of Aug. Lumière that plants even may be susceptible to anaphylaxis.

In man shock may follow the reinjection of a therapeutic serum. It may be well here to recall what we have said regarding serum disease as it follows a *first* injection. This condition appears, as we have seen, only after an incubation period of from seven to twelve days. But circumstances may require a second injection of serum after a greater or less interval. The first injection, for example, may have been diphtheria antitoxin administered therapeutically in diphtheritic infection. Some months, or some years later, the same person is affected with dysentery, with cerebrospinal meningitis, or suffers an injury which renders tetanus a possibility. He is then injected with antidysentery, antimeningococcus, or antitetanic serum. All of these sera are, however, derived from the horse. After a second injection the reactions to the serum appear after a period which is the shorter as the interval of time elapsed since the first injection is the less, and these reactions are apparently the same as those resulting from a first injection—fever, joint pains, urticaria. This is, at least, the case when the injection is given by either the subcutaneous or intramuscular route, as such therapeutic sera are usually given. These avenues of introduction are employed for the purpose of avoiding a sudden entrance of the foreign albumin into the circulation, thus attenuating very greatly the severity of the shock. But if the second injection is given by the intravenous route, as is done under some circumstances,[2] a typical, often violent, anaphylactic shock follows.

[2] It is sometimes necessary, because of the urgency of the case, to give the serum intravenously. In such cases the method of desensitization of Besredka should be applied, thus removing the risk of accident

THE CAUSES OF SHOCK

Why this difference in the length of the incubation period—seven to twelve days in the case of a first injection, and a few hours, or three, four, or five days according to the elapsed time, after a reinjection? Von Pirquet and Schick gave the first interpretation, and the facts since discovered demonstrate its correctness. The foreign albumin introduced into the body, especially when in a dose of 10 or 20 cc. or even more, as is the case with therapeutic sera, is eliminated but slowly. Twelve to fifteen days after the injection it is still possible to reveal its presence in the blood.

From quite a different point of view we have seen that all albuminous colloids, if heterologous, lead to the formation of antibody. And serum disease occurs at the precise moment when this sensitizer appears in the blood. Anaphylactic shock is a result of the union of this antibody which has been formed with antigen which still persists in the body. So much for the accidents which follow a first injection. If the second injection is given when the sensitizer developed by the first injection is still present in the blood the reaction appears after a few hours only. If the first injection antedates the second by a long time (one or several years) the reactions are delayed, because, as experiment shows, the body is required once more to produce an antibody. But during this interval it has conserved a special aptitude to produce it anew under the influence of a second injection, hence the period of incubation is shortened and this in proportion as the time of the first injection is more or less distant. Thus it is that in such cases the antibodies appear and the reactions occur within from one to eight days, according to the time elapsed since the first injection.

It is clear, then, that it is the antigens, and the antigens solely, which are capable of sensitizing an animal and of eliciting anaphylactic shock. The strict specificity can likewise be understood. A reinjection is not intoxicating except it be made with an antigen of the same type as that which produced the sensitization. The quantities required to sensitize are precisely those which are sufficient to provoke the formation of antibody. And, finally,

the incubation time necessary for sensitization is exactly the time required for the formation of antibody.

Moreover, passive anaphylaxis is conclusive in this regard. Incite in a rabbit the appearance of sensitizer by a series of injections of a given antigen, sheep serum for example. About twelve days after the last injection remove 1 to 2 cc. of blood from the rabbit and inject it into the vein of a normal guinea-pig. Test this guinea-pig a few hours later by an intravenous injection of sheep serum. It will show the anaphylactic crisis, and perhaps die. This guinea-pig is, therefore, sensitized against sheep serum without a long incubation period, and this is true because the rabbit serum with which it was injected contained the antibody specific for the albumins of sheep serum. Such sensitization is called passive, for the specific antibody is formed in the body of another animal. Needless to say, some eight to ten days later the guinea-pig becomes sensitive to rabbit serum, but here actively, since it has itself formed antibodies to the albumins of this serum.

Take a rabbit serum which contains antibodies specific for the albumins of sheep serum. Combine it with sheep serum in vitro. We will obtain, as we know, both the fixation of the sensitizer to the albumins of the sheep serum and the flocculation of these albumins. The rabbit serum is deprived then of its antibodies which are fixed to the corresponding albumins. A serum so treated no longer produces passive sensitization, an evidence that anaphylaxis occurs only by virtue of the antibodies.

The anaphylactic crisis is due, therefore, to the union within the body of an antigen and its specific antibody. Upon this everybody is in accord, for experiment does not allow of any other interpretation. But the unanimity of opinion disappears when the result of this union becomes subject to discussion.

THEORIES OF ANAPHYLAXIS

Individually, antigen and antibody are inoffensive. But in uniting, whether it be in vitro or in the body of an animal, they acquire the property of bringing about a reaction. Thus, Friedmann and Friedberger have concluded that in the course of this reaction an anaphylatoxin is formed.

But this supposed anaphylatoxin can be obtained without the intervention of antibody; the only essential is the presence of complement. Friedberger himself has shown that if bacteria of any kind whatever are suspended in *fresh* serum, the supernatant fluid, after removal of the bacterial bodies, contains an anaphylatoxin. Can it be obtained without the intervention of bacteria? It is only necessary to take red blood cells, or, it is even sufficient to mix in suitable proportions two sera derived from animals of different species; and, finally, Bordet obtained anaphylatoxin by adding to *fresh* serum a trace of agar,[3] melted by heating in physiological saline.

It is significant that anaphylatoxin is always formed only with fresh serum. If serum be but only a few days old anaphylatoxin is not produced. Complement is therefore indispensable. Friedberger assumed that the complement exercised upon the antigen a decomposing action, a digestion, leading to the production of a toxic compound. To this interpretation there is but one objection, but it is important and is adequate to cause its rejection, namely, that complement is not a ferment and has no decomposing action upon the albumins. Furthermore, it would indeed be strange if all albumins, whether they belong to a bacterium or to a red blood cell, whether they are derived from a plant or from a man, all produced an anaphylatoxin endowed with identical properties and always eliciting an identical anaphylactic crisis.

According to Bordet the anaphylatoxin is not derived from the antigen itself, and he offers as proof the fact that an intravenous injection of a suspension of agar is sufficient to cause a typical anaphylactic crisis.

This last observation allows us to see what the anaphylatoxin really is.

THE MECHANISM OF SHOCK

Very recently, Mlle. P. Mendéléeff has investigated by means of electrometric methods the nature of the modification imparted to a serum by the addition of agar. She has determined that the

[3] An extractive product of certain algae of the China Sea. It has the property of forming a gel upon cooling.

concentration of free H^+ ions of fresh rabbit serum is 1.58×10^{-8}; for the same serum after the addition of a minute amount of agar it is 3.98×10^{-7}; and for a double addition of agar, 2.5×10^{-5}. The addition of agar, then, renders the serum strongly acid, and the concentration of free H^+ ions corresponds to the isoelectric point of the albuminous colloids of the serum, that is to say, to the *point of coagulation*.

She has likewise proved that the phenomenon follows the same process in vivo as in vitro. She has found the same augmentation in free H^+ ions in the serum of an animal immediately after an injection of serum. She has demonstrated experimentally that the introduction into the blood of the animal of serum once treated with agar is equivalent to a second treatment with agar in vitro, and brings all, or part, of the blood proteins to the isoelectric point which corresponds to their flocculation.

In addition she has shown that the tendency to flocculation can be obtained only by the treatment of *fresh* serum with agar. The addition of agar to a serum heated at 56°C. makes the content of H ions vary, but does not bring the serum to the isoelectric point. And in another place we have stated that heated serum treated with agar does not produce anaphylatoxic shock in animals.

And finally, she has shown that the same phenomenon takes place when the shock is induced by a reinjection of a protein. It takes place in all cases where H^+ ions are liberated. Consequently the serum proteins of the animal in a state of shock tend to approach the isoelectric point.

She has concluded[4] that the intimate processes of anaphylaxis appear to be based upon three phenomena:

1. Modification of the physico-chemical constants of the blood, the cause or the effect being associated with oscillations in the pH of the blood serum.

2. Modifications in the cellular permeability, which, permitting a change in the physico-chemical equilibrium of the cellular contents, lead to disturbances in the structure of the protoplasm and in the physiology of the tissues.

3. Physiological manifestations of these intracellular disturbances reflected in an abnormal working of the organs.

[4] Compt. rend. Soc. de biol., 1924, **90**, 602.

As has been indicated in the book *Les Défenses de l'Organisme*, the sudden liberation of H ions in the blood, and consequently into the internal medium which bathes the cells, by itself explains the cellular manifestations. Any cellular modification involves naturally as an immediate result a modification of cellular metabolism which is reflected in the functioning of the organ. In brief, all of the phenomena which take place in shock result naturally from the effects of the liberation of H ions in the blood. Needless to say, this statement does not exhaust the question, for it is now necessary to solve the following problem: What is the mechanism of this liberation of H ions which takes place at the moment when sensitizer unites with the antigen? This is a problem of colloid chemistry.

The experiments of Zunz lead to the same conclusions as those of Mlle. Mendéléeff. Zunz bled his guinea-pigs at the height of anaphylactic shock, separated the plasma, and showed that "the reduction in surface tension parallels the violence of the shock. The alkaline reaction of the plasma is diminished, and even at times, the plasma may become slightly acid. In general, the changes in surface tension parallel the reaction of the plasma."[5]

Zunz repeated the same experiments with guinea-pigs passively sensitized by an injection of the serum of guinea-pigs anaphylactic to horse serum (that is to say, previously treated by an injection of horse serum). Intravenous injection of these passively sensitized animals with variable quantities of horse serum induced the following changes in the serum.[6]

QUANTITY OF HORSE SERUM INJECTED	SYMPTOMS	APPEARANCE OF THE BLOOD	pH	SURFACE TENSION PER SQUARE CENTIMETER
cc.				*dynes*
0	None	Red	7.38–7.43	74.8–75.6
0.3–0.4	None	Red	7.32–7.46	74.7–75.7
0.4–0.5	Slight	Red	7.36–7.40	73.6–74.8
0.5–0.6	Severe	Venous	7.28–7.33	72.2–73.2
0.5–0.6	Violent	Venous	7.16–7.18	70.9–72.1
0.5–0.6	Very violent	Very venous	6.88–7.12	68.7–71.1
Normal pigs			7.35–7.48	74.2–76.6

[5] Kopaczewski was the first to observe this reduction in surface tension.

[6] Compt. rend. Soc. de biol., 1924, **90**, 650.

These facts offer much by way of explanation. The "anaphyla-toxin" is represented by the liberation of free H ions which cause the serum to become acid. It can be understood, then, why shock always manifests itself in an identical fashion whatever may have been the causative protein, since the crisis is always the result of a sudden break in the equilibrium of the colloidal state of the blood, with the liberation of free H ions.

The anaphylactic reaction, which unfolds always according to an unchanging plan, is necessarily provoked in all cases by a similar cause. This cause is always the disturbance of the colloidal equilibrium by a liberation of positive ions such as is always produced in the antigen-antibody reaction.

Many authors, of whom Kopaczewski was the first, have suggested that the flocculation of the serum proteins under the influence of the antibodies might constitute the initial cause of the crisis. That such a flocculation occurs can not be disputed, and we now know the reason, but the flocculation alone is not sufficient to explain all of the disturbances, and in particular the very peculiar state of the lung characterized by rigidity of the pulmonary tissue. These disturbances are, in reality, provoked by the liberation of positive ions. It is evident, in fact, that the sudden appearance in the blood of these ions has of necessity an immediate repercussion upon all of the cells of the body, and in particular upon the endothelial cells of the vessels; and the tissues most abundantly supplied with blood are the pulmonary and hepatic tissues. There is produced necessarily a tendency toward coagulation of the cellular protoplasm, which explains, for example, the rigidity of the pulmonary tissue such as is observed in animals which die of shock.

To such a theory the objection may be made that if it is indeed the union of sensitizer and the antigen which leads to the liberation of H ions it is fair to assume that, in guinea pigs which have received the same quantity of sensitizer (because they have been passively sensitized by the same quantity of serum derived from a single sensitized guinea pig, as in the table above) the quantity of H ions liberated ought to be proportional to the quantity of antigen injected to provoke the shock. Accepting this, how is it that a guinea-pig receiving 0.4 cc. of horse serum failed to

react at all, and that in parallel its blood serum failed to change in pH, while another pig prepared in the same manner, receiving only an additional tenth of serum, 0.5 cc., died of the reaction?

But it must not be forgotten that the blood exhibits the phenomenon of automatic regulation, tending to maintain fixed the quantity of H ions, thus controlling the ionic equilibrium of the blood colloids. As we have seen, this instantaneous autoregulation is assured by the buffers, and then, more slowly, by an exaggeration of the respiratory elimination of carbonic acid.

The symptoms and lesions of shock are not determined by the total number of H ions liberated in the reaction between sensitizer and antigen, for a portion of these ions are immediately fixed by the buffers. The cause of shock is the *excess* of ions which remain free after the play of the buffers. Obviously, this excess may be variable for a single animal at different times, and for different animals of the same species at the same time, since it depends upon the ionic state, and especially upon the state of the buffers, at the moment when the antigen-antibody reaction is effected.

In a word, if the conditions in the blood at the moment of the antigen-antibody combination are such that the liberated H ions are immediately fixed, no variation in pH occurs and shock does not take place. But it does occur if the buffers are inadequate, and the intensity of the shock is in direct proportion to the number of ions which remain free.

It is clear, then, why shock may be avoided if care is taken to introduce the intoxicating injection slowly. The H ions are liberated gradually, permitting not only the buffers to function, but also the other phenomena which regulate the ionic equilibrium of the blood.

Further, it is possible to understand that if the shock is not fatal within a short time, all of the disturbances may be dissipated without leaving a trace. The ionic equilibrium of the blood is quickly reëstablished if the coagulation of cellular protoplasm, under the effect of the transitory hyperacidity of the blood, has not reached the point where it becomes irreversible. The rapid reëstablishment of the normal alkalinity leads automatically to the return of the protoplasmic gel to its normal state.

Under certain circumstances the irreversible coagulation of the protoplasmic gel does not involve all of the cells of an organ, as is the case when lethal doses are used.   In such a case irreversibility involves only a limited number of cells, leaving the organ still capable of functioning to a certain extent.   Death may then intervene sooner or later, due to a functional insufficiency, most frequently of the liver.

On the other hand we must avoid the error of assuming that reactions which may take place within the blood can take place in this tissue only.   The organism is a complex colloidal system, of which all of the parts are continually in equilibrium.   It is impossible to conceive of a sensitizer existing in the blood and not being found in intimate contact with the cells by way of the interstitial fluid.

The reaction may take place, then, in the region of the cells. Indeed, the experiments of Dale upon isolated organs show beyond any possible doubt that the cells of an hypersensitive animal are of themselves susceptible to shock.   But it is probable that in the case of all cells the process takes place not *within* the cell, but in the region of the cell, that is, upon its surface, since the colloidal antigen certainly can not penetrate a cell which does not possess the phagocytic property.   The cell undergoes at its periphery a reaction of flocculation.

On the other hand, the sensitizer, which perhaps might better be termed sensitizin, can not be a product of humoral origin.   Its place of formation, as we will see, is most probably the endothelial cell of the capillaries and this elaborating cell ought to participate in the shock.

It would appear that we might represent the phenomenon of anaphylactic shock according to the following scheme.

Antigen gaining access to the circulation reacts first with the sensitizer free in the blood, whereupon free H^+ ions become liberated.   The immediate result is, according to the quantity of antigen introduced and according to the state of the buffers, a partial or complete flocculation of the less stable micellae of the blood, that is, of the fibrinogen micellae.   This, in turn, results in a greater or less incoagulability of the blood and also in the formation of thromboses within the capillaries.   Where there is

an excess of H^+ ions the flocculating action extends to the cells bathed by the blood, and it is at this time and under these conditions that the shock actually becomes manifest.

If the quantity of antigen is sufficient, to this initial phenomenon is added a second, in which the reaction takes place upon the elaborating cell itself, impregnated as it is with sensitizer. This cell undergoes a profound modification, expressed by a flocculation of its protoplasm, with modifications in the electrostatic charge, in the surface tension, and in the permeability of its peripheral zone. As a result the capillaries themselves are modified (vasomotor changes with the tendency toward hemorrhage) leading to a derangement in the functioning of the organs, chiefly in those to which a perfect function of the capillaries is essential to activity, particularly the lung and the liver.

Quite aside from anaphylactic shock, properly speaking, of which the immediate cause is an antigen-antibody reaction, each time that there occurs in the blood a sudden liberation of positive ions, through some colloidal reaction, there will be a crisis of the same order. Such is the case in simple protein shock. The immediate cause of the diverse shocks may be very different, the ultimate cause only is always identical, and it is because of this that we always have the same symptoms.

NATURE OF THE SENSITIZER

The essential character of antibody is its specificity. Let us state, however, that this specificity is strict only in so far as it concerns the proteins which are found in the form of micellar complexes. It does not exist for the proteoses, as Zunz has shown. But in so far as the complex proteins are concerned the specificity is strict; the reaction can occur only between a sensitizer and a protein substance identical with that which led to the formation of the sensitizer. "The colloidal chemistry of the formation, and in particular of the specificity, of antibody is the greatest wonder of the world" (Sahli). It is necessary to assume, so to speak, an infinite number of possible protein micellae, and a corresponding number, likewise infinitely great, of sensitizers.

To explain this specificity diverse and strange hypotheses have been advanced. It is impossible to dwell on them. Only that of

Sahli will be mentioned, which supposes the inherent and normal presence, physiologically, of all possible antibodies preformed in the blood.

Without desiring to advance a theory, it would seem that the following might be considered as probable:

1. That the sensitizers are substances and not properties. Such a property could only be a state of colloidal equilibrium. But sensitizers are stable; they persist unaltered despite changes in states of equilibrium in the blood plasma. Complement is a state of equilibrium; the "alexic state," obtaining only for a given equilibrium of blood serum. When this equilibrium is not realized in the circulating blood, or when it is destroyed, as is the case normally by the ageing of serum for some days, the alexic state disappears at the same time as the equilibrium of which it represents a property.

2. The sensitizers must be elaborated by the cells, and the following considerations permit us to infer what cells are involved.

a. Only an albuminous colloid is able to cause the formation of a sensitizer.

b. The only cells into which such colloidal substances are able to penetrate are the phagocytic cells.

c. If an animal is repeatedly bled it can be shown that the sensitizer quickly reappears. The elaborating cell can not, therefore, be an ameboid phagocytic cell of the blood itself.

d. The elaborating cell must, then, be a fixed cell having the power of phagocytosis, located most probably in close association with the circulatory system.

The place of formation of the sensitizers, which might better be called sensitizins, is, therefore, most probably the endothelial cells of the blood capillaries or of the lymphatics.[7]

CONCLUSIONS

From all of the above statements and deductions we may conclude:

1. That the antigen-antibody reaction is accompanied by a liberation of H ions.

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[7] Indeed, Cary (J. Med. Res., 1922, **43**, 399) has already suggested that the "hemolysins" must be elaborated by the fixed phagocytic cells of the tissues.

2. That this liberation of H ions takes place principally in the circulating blood, and then upon the endothelial cells of the capillaries.

3. That the buffers of the blood take up, according to the conditions of the moment, either all or a part of the ions liberated. If free ions remain shock occurs, and this to a degree proportionate to the excess of free ions.

4. That by virtue of an excess of free H ions in the blood there occur modifications in the state of equilibrium of the cellular gels in the direction of a coagulation. These cellular modifications, occurring indirectly under the influence of the free H ions of the blood together with changes undergone directly by the endothelial cells represent the significant changes of anaphylactic shock.

5. The excess of free H ions exercises upon the elements of the blood itself the same coagulating action as upon the cells bathed by this blood. A flocculation of the less stable albuminous micellae, that is, the micellae containing that which we term fibrinogen, takes place.

6. These floccules may produce by themselves the particular effects of thrombosis, as several authors have suggested, or even an exciting action, according to the theory of Lumière. The excitation by these flocs of the nerve endings in the vascular endothelium within the nervous centres provokes a sudden vasodilatation of the visceral vessels and the fall in arterial pressure. This last action does not represent, as Lumière believes, the most important process. It is only one of the results of the liberation of H ions. It may be the cause of one of the multiple symptoms of shock, but the chief one is cellular.

To re-state some of the facts; we have seen that, contrary to all advanced hypotheses, the fixation of complement does not lead to a dissolution, to a lysis, but to a coagulation, as examination of the stroma of the red blood cells shows. We can see now that it can not be otherwise, since the fixation of complement is accompanied by the liberation of positive ions, and that the presence of these ions is precisely the cause of the flocculation of albumins.

There is but a single antibody, the sensitizer, which in all cases, both in vitro and in vivo, brings about a coagulation.

THE THEORY OF "LYTIC" SERA

If there is a history which presents a philosophical significance from the point of view of that which may be called "the history of an error" it is indeed the history of the "antibodies."

In 1894 Pfeiffer and Issaeff observed the granular transformation of vibrios in the peritoneum of prepared guinea-pigs. They christened this transformation "vibriolysis," meaning a dissolution of the vibrios. From that time on many investigators attempted to reproduce this phenomenon with the most diverse bacteria, but without success.

In 1898 Bordet reproduced the phenomenon in vitro, and discovered that it was effected under the influence of two factors, sensibilisatrice or sensitizer and complement or alexin.

The phenomenon became the object of innumerable investigations, which demonstrated that following the introduction of an albumin the body responds by the production of a specific sensitizer which is electively fixed by an albumin of the same type as that which was injected.

The sensitizer became a "lysin;" the simple fixation of complement to a bacterium, in which nothing further is involved, became "bacteriolysis," and the serum containing the sensitizer must be a "bacteriolytic" serum.

The magic of words. No one had ever observed the bacteriolysis of a single bacterium under the influence of a serum, but nevertheless, since then many impassioned controversies have been waged to explain the mechanism of this non-existant phenomenon. Various theories have been evolved assigning "bacteriolysis" as the very basis of immunity. This bacteriolysis which leaves the bacterium living and endowed with all its properties has become an intangible dogma, accepted by all, and which has weighed heavy for more than twenty years upon the science of immunology.

As a matter of fact, the formation of antibodies takes place every time that a foreign albumin is introduced into the tissues of the body, although it is indeed probable that they do not function as substances in the strict sense of the word, but as modifications in the equilibrium of the colloidal state of the organism. But how-

ever this may be, the antibodies do not provide any processes of defense against the bacteria; they function solely as a reaction of colloids upon colloids.

We will see later that the entrance of bacteria into the body does provoke true specific antibacterial reactions, but the bacterial cell being constituted of colloids, the *antibacterial* reactions are accompanied necessarily by the production of *anticolloid* antibodies, which are the anaphylactic principles, that is to say, the "contra-protecting" principles. Antibodies can only be considered as the fatally expensive price of immunity.

PART THREE

THE DEFENSE AGAINST ANIMATE AGENTS

CHAPTER I

The Bacteria

THE STRUGGLE AMONG LIVING BEINGS

All living beings withdraw from their environment the elements essential to the maintenance of life, and, as the quantity of these elements is not limitless the competition is keen and each must struggle to appropriate the foodstuffs required.

All animals are obligatory parasites, since they are forced to borrow from other living beings the organic matter which they can not elaborate for themselves. It is not so among plants, which, deriving their energy from the light rays, are not forced to struggle to the same extent among themselves to reserve to their own use the small portion of ground from which they derive their nourishment.

In this struggle the being attacked reacts always, and the reactional processes employed are innumerable. Each species of animal, and even of vegetable, has those peculiar to itself. The plants, the insects, and the reptiles secrete toxic fluids. Certain fish possess organs which produce electricity. Some marine animals emit fluids which cloud the water. Certain insects are able to free themselves after capture by detaching the captured member. Since a review of all of the particular defenses would go beyond the limits of our subject, we can only include the general defensive processes, common to all living beings, to vertebrates in particular, and we will dwell more particularly upon these facts as they relate to man.

Against the parasites most to be dreaded, the smallest of all living beings, the bacteria, the means of defense of the body are identical in all types of animal. Finding in the bodies of animals an environment favorable for their development, the bacteria withdraw from this medium the elements necessary for their growth; they multiply. This bacterial invasion of the body of the animal naturally disturbs the functioning of its organs and the

animal becomes sick. But this animal does not submit passively to the attack of the bacterium. It reacts.

Before examining the means of defense of the animal, let us review briefly the army of invaders, the microörganisms, and see what means of attack they possess.

The agents of infectious disease may be divided into three classes, the Protozoa, the Protophytes, and the Ultraviruses. Inasmuch as the types of reaction which the last of these classes provoke are distinct from those induced by the visible parasites— the Protozoa and the Protophytes—we will not consider the viruses here but, because of their great importance, we will reserve a consideration of them until later (Part IV).

THE PROTOZOA

The protozoa are unicellular beings belonging to the animal kingdom, and although several dread diseases, such as malaria, sleeping sickness, and amebic dysentery, affecting the higher vertebrates may be caused by such parasites we need hardly consider them here, for up to the present it has been impossible to cultivate the protozoa in artificial media and because of this fact experiment becomes exceedingly difficult. All that may be said, is that certain observations show that the means of defense against the protozoa are the same as those employed in the case of attack by bacteria, phagocytosis playing a very important rôle.

THE BACTERIA

The protophytes are plant, just as the protozoa are animal, unicellular beings. The parasitic species do not form a definite family but are, on the contrary, distributed throughout an infinity of other species which are absolutely inoffensive since they develop solely at the expense of dead matter.

Some species of the lower fungi are capable of living at the expense of the higher vertebrates but the agents of the most dreaded infectious diseases are to be found gathered in a single, although poorly defined, group—the bacteria.

Bacteria are abundantly distributed throughout the external environment of animals; the air, the water, the soil, the smallest

particle of organic matter, is peopled with an infinity of bacteria. These innumerable bacteria of our environment appertain to various species, a considerable number of which have already been described and new ones are found each day, but it is certain that the number of those which remain to be discovered is much greater yet.

Classification of these species is difficult. Basing the classification only upon form, they are grouped in families, the cocci presenting the aspect of minute spheres, the bacilli of little rods, and the spirilla appear as small curved elements, shaped like commas, parentheses, or often forming a spiral of several turns. The Leptothrix forms long straight filaments, the Cladothrix produces filaments which are branched. Sometimes it is very difficult to classify a bacterium in any one of these groups, for, according to the circumstances of the environment, it may take one form or another. The tubercle bacillus, for example, has ordinarily a bacillary form, but it may assume the form of a cladothrix. Internal structure is of no service in differentiation, for it appears to be the same in all species. The size of these beings, a few thousandths of a millimeter in the greatest dimension, is such that structure can not be defined.

Certain bacteria possess very fine cilia; they are motile. Others do not have them, hence they are non-motile. All degrees of motility can be observed between an extreme motility and an absolute lack of motility. Even in a single species certain varieties are motile, others are but slightly motile, and still others lack the power of motion entirely. Motility is not, then, a fixed character.

The bacteria, like all living beings, have an absolute need of oxygen. Certain forms derive it directly from the air dissolved in the medium in which they live, whereas other forms are unable to develop in the presence of free oxygen but must borrow it from oxygen-containing compounds. The first are termed aerobes, the second anaerobes. Many bacteria are aerobic-anaerobic, that is to say, that in the presence of air they utilize the oxygen directly, and in the absence of air they comport themselves as anaerobes. There is much debate on the mechanism of the harmful influence exercised by oxygen upon the anaerobic bacteria. It is probable that this action is exerted upon the ferments which regulate their

metabolism, all ferments being more or less sensitive to the action of oxygen. A ferment readily oxidizable can not act, obviously, in the presence of free oxygen, as must be the case with the ferments of the strictly anaerobic bacteria. This would explain why they are unable to develop in the presence of air, or at least why they are found multiplying only in an environment containing reducing substances, that is, substances capable of binding oxygen.

BACTERIAL REPRODUCTION

As a general rule, whenever a bacterium is placed under conditions normal for its existence, when it finds in its environment an abundance of the elements necessary to its existence, it multiplies by a process of simple division. The bacterium divides into two parts, each of these parts increases in size and then they, in turn, divide into two portions, and so on. This division takes place rapidly, expecially in a new medium, that is, when the number of organisms present is limited. The interval of time which separates two divisions may then be only a few minutes.

In a number of species this simple divsion may give place to the formation of spores which appear only when the medium becomes unfavorable for growth, either because of too small an amount of food substances or because of the accumulation in the medium of harmful substances composed of the waste products of the metabolism of the bacteria. The spore is a small spherical or oval body surrounded by a membrane formed of the condensed protoplasm. In general, the body of the bacterium disintegrates immediately after the formation of the spore, the latter remaining free in the medium. In brief, the spore resembles a seed. It resists the destructive forces of the environment much better than does the vegetative form of the bacterium. Many spores, immersed in water, can withstand for several minutes a temperature of 110°C., when dry they are killed only at about 140°; while the vegetative form is deprived of life by subjection for only a short time to a temperature of about 70°C. It should, however, be mentioned that certain bacteria are able to develop normally at temperatures approximating 95°C. Such are the thermophilic bacteria which live in hot springs.

BACTERIAL NUTRITION

Like all living beings, the bacteria must procure from the environment the elements which furnish them both with the materials destined to the building up of their own tissues and the energy which allows them to effect this synthesis.

The alimentary needs of the different species of bacteria are very variable.

Certain bacteria, studied by Beijerinck, when found in a sulfur-containing medium, synthesize the amino acids and build up the protoplasmic micella by utilizing ammonium nitrate as a source of nitrogen, sodium carbonate as a source of carbon, and water as the source of oxygen and hydrogen. These bacteria lack pigment and do not derive their energy from light but procure it through the decomposition of hydrogen sulfide or of alkaline sulfates. The nitrifying bacteria of the soil borrow the carbon from the carbonates, the decomposition of which also provides them with energy. Nitrogen is obtained directly from the air. Other bacteria likewise withdraw nitrogen from the air, but energy is supplied only by the decomposition of ternary substances—sugars, starch, glycerin, alcohol, etc. Finally, others must withdraw all of the elements necessary to them from already formed organic material. Among these are the bacteria which develop exclusively at the expense of dead matter, the saprophytes. Others, that is, those forms which live only as parasites in the tissues of another living being are able to multiply only at the expense of living matter. And finally, certain forms are able to develop indifferently upon either dead or living matter. These are the facultative parasites.

PARASITISM

The problem of how certain bacteria have become parasites has occupied savants for a very long time. It might even be said that the concern of thinkers on this subject is a concern of the past, together with the riddle of the egg, which is so often met with in the scholastic discussions of the middle ages: The chicken or the egg, which gives birth to the other? Did rabies antedate the dog, or the dog, rabies?

In so far as parasitic bacteria are concerned there is still another manner of asking the question—Is a bacterium able, under certain particular conditions, to become a parasite? This question is very important, for if it is answered in the affirmative, new contagious diseases, previously unknown, may suddenly make their appearance. It would seem that, in fact, this may take place. With regard to bacteria there are certain experiments which tend to support this belief, but these experiments are not entirely free of criticism. This is not true of the experiments which show that saprophytic fungi may become parasitic for plants. The experiments of Massee are particularly interesting in this respect, for they show, in addition to the actual fact, the mechanism of the adaptation.

Massee first showed that the infectious characteristic of microscopic fungi is due to the presence of substances, possessing a positive chemotaxis, in the cells of the host. He next showed that all of the saprophytic fungi, unlike the parasitic ones, have a positive chemotaxis for glucose. He then injected into the tissues of the plant a solution of glucose, and saw that a saprophytic fungus penetrated into the tissues impregnated with the chemotactic substance, and behaved there like a parasite.

He took, for example, *Torula herbarum*, a banal saprophyte. The sugar he injected into the tissues of the leaves of begonia. The Torula, deposited on the leaves, penetrated into the tissues and multiplied there. Thus several passages were effected, diminishing with each transfer the amount of the sugar solution injected. Gradually the Torula adapted itself; the positive chemotaxis which it possessed for the sugar little by little diminished, and at the same time a positive chemotaxis for the juices of the leaves developed. Finally, the Torula acquired completely the pathogenic character and invaded the tissues of normal leaves of the begonia. Let us note that the pathogenic character is transmitted to its descendants, for, in the course of these experiments the Torula under experimentation was subdivided a great many times, and despite this the adaptation was progressive.

It is by no means impossible that the experimental conditions of Massee might not at times be naturally realized in nature. We must in any case admit that a saprophyte can, by adaptation,

become parasitic, and that, as a consequence, new diseases may make their appearance upon the earth at any given moment. It is very probable that this has occurred many times since life made its appearance upon the globe, and that the disappearance of many species, animal and vegetable, was due to the adaptation of a bacterium to parasitism for these species. Let us remark that such new diseases must be extremely fatal, for the individuals of the species to which the adaptation is accomplished can not possess an acquired hereditary immunity. But, on the other hand, a microörganism perfectly adapted to its host can develop only under the strict conditions to be found in this host and such as are never found elsewhere. Numerous known bacteria are in this condition; we might cite the gonococcus, the meningococcus, and the spirochete of syphilis, among others.[1] If a bacterium of this type increases its virulence to a degree such that the host is no longer able to oppose an adequate defense the host will die, and the animal species parasitized by such a bacterium will be eliminated, the elimination involving equally the parasitic bacterium, since an obligate parasite can only develop in the body of its habitual host. This has doubtless happened many times in the course of geologic time, and it is possible that the bacterial diseases which existed in the tertiary epoch were not the ones which now obtain among the diverse species of animals.

[1] Adaptation to parasitism with the protozoa is always very strict, but here the phenomenon is complicated. Biology reveals a great number of protozoa possessing a peculiarity which, it is necessary to say, is really inexplicable. The cycle of their complete development is effected in the bodies of two different animals, of very different species. As examples—the trypanosome of sleeping sickness is only able to accomplish its cycle of evolution in the tse-tse fly of the Glossina genus, and in man. It passes alternately from the one to the other. The evolutive cycle of *Piroplasma bigeminum*, the agent of Texas fever in cattle, commences in the ticks and ends in the cow. One might say that here the situation is still more complicated, for the piroplasma which develop within the blood of the cow as the result of the inoculation of the parasite by a tick, are absorbed with the blood sucked out by another tick, and are not inoculated into another cow by this tick but only by the daughters of this tick. Comparable facts are noted in connection with many species of protozoa, and for certain parasites higher in organization.

In nature facultative parasitic bacteria are rare. Few of them actually multiply in the external world, but more usually grow in the intestinal canal. When thrown out into the exterior they do not multiply. But, while some of them are able to cause disease if they are ingested by a receptive individual, others become pathogenic only under special conditions. The spore-forming organisms which cause disturbances following trauma, the tetanus bacillus, *B. perfringens*, and others, live without causing any trouble whatever in the intestine of man and of many animals. It is only when they are introduced into the damaged tissues that they provoke disease—tetanus, gaseous gangrene, etc.

The obligate parasites, on the contrary, seem to be able to live naturally only in a tissue. The term *naturally* is used advisedly, since, for most of them it has been possible to prepare artificial media in which they will multiply. But these necessary conditions of the medium are produced only experimentally, and are never met with in nature.

In general, then, apart from rare exceptions, all pathogenic bacteria are in reality obligate parasites, which is to say that *in nature* they need for their development the special conditions to be found only in a living being; that they vegetate, according to circumstances and perhaps alternately in the digestive tract and in the tissues, or they may be able to multiply exclusively in the tissue.

THE CULTIVATION OF BACTERIA

As we will have to consider at different times experiments dealing with the cultivation of bacteria it may be well to mention briefly certain facts concerning the methods employed in bacteriology.

The growth of bacteria is obtained artificially either in liquid media or on solid media. The basic medium is a meat bouillon containing peptone. This peptone bouillon serves for the cultivation of the majority of bacteria, although for certain delicate forms there is added some appropriate substance, such as albumin (serum, or egg white), sugar, glycerin, etc. The solid media are obtained by preparing a gel with the peptone bouillon by the addition of gelatin or agar. Solid media are distributed into tubes

or into flat dishes, Petri plates, while the medium is still warm and fluid. It is sterilized at 115°C. and the tubes or plates are allowed to cool, the tubes in an inclined position so that after cooling a layer of the gel is obtained.

Cultures of bacteria for experiment must be pure, that is, uncontaminated with any organism other than the one desired for study. But, a bacterium is rarely found unmixed with associated bacteria in a product from which its isolation is desired. To effect this isolation the product is emulsified carefully in a sterile liquid and then small drops of this emulsion, sufficiently diluted, are spread over the surface of the agar in a tube or plate. When the bacteria in the dilution are few each of them is deposited during the inoculation well separated from its neighbors. The tube or plate is placed in the incubator, each bacterium multiplies, and forms a colony which gradually increases in size until after a certain time, twelve hours to two or three days, it becomes visible to the naked eye. This colony is formed solely of individuals of the same bacterial species, since they are all the issue of a single cell.

A portion of such a pure colony is taken by means of a platinum wire (the wire having been heated red hot in the flame to kill the germs of the air which may be adherent to it, and cooled immediately before use) and transferred, either to a tube of liquid medium or to the surface of nutrient agar. In the first case, the bouillon becomes turbid within a few hours as a result of the multiplication of the bacteria inoculated; in the second, if the surface of the agar has been rubbed over with a portion of a colony one obtains after some hours a uniform layer, more or less creamy in character, composed solely of the young bacteria resulting from the multiplication of those which were inoculated. These cultures may be continued serially for an indefinite period, always in a state of purity providing a suitable bacteriological technic is observed.

The aspect of the cultures, the biochemical reactions which can be effected with them, the amount of acidity or alkalinity produced, the detection of different products, such as toxins, liberated by certain species of bacteria, etc., permit the characterization of the bacterium. Furthermore, these cultures may be inoculated into experimental animals and the disturbances pro-

voked, variable according to the species of the bacterium, serve likewise to establish a diagnosis.

These statements are necessarily schematic—isolation is often much more laborious, the medium necessary for growth may be much more complicated—but such as they are, they will permit a more ready comprehension of certain statements which will appear in the chapters to follow.

CHAPTER II

The Conditions of Infection

GENERAL CONDITIONS

Daily observation shows that, in relation to a given bacterium certain animal species are susceptible, others are refractory, and that, within the susceptible species, a first attack of the infectious disease, if it is not fatal, may protect the individual, for a variable length of time, against a new attack of the same disease.

Immediately a question presents itself. Having given in the same environment a susceptible individual and a bacterium pathogenic for the species to which this individual belongs, is infection inevitable? So it was thought at first, but Pasteur was not long in perceiving, as he published in several treatises, that it was necessary for certain conditions to be realized in order to have infection take place. The more advanced our knowledge becomes, the more it appears, in fact, that while the environment swarms with bacteria pathogenic for different animal species, nevertheless relatively, infection is rare. A study of the conditions which permit infection, disease, to occur form a preface to those of immunity properly speaking.

The process of infection is extremely complex, much more so than has been thought up to the present time. It depends not only on the respective properties of the two beings, the animal and the bacterium, but always on three, often even on four living beings, as will be shown in the following chapters. For the moment, let us go from the simple to the more complex, let us consider the case of the two beings of the greatest size, two visible beings found together, the animal and the bacterium.

In biology nothing is absolute. This fact has already been insisted upon, and it must be continually held in mind if we would understand even the most simple facts. All of the conditions of the environment influence the living being. Adaptation is continually taking place and the mark of these successive adaptations

is left upon the substance. Every living organism throughout the course of its existence is exposed to different conditions, and the sum of the characters gradually acquired added to the sum of the equally special characters inherited is peculiar to each individual. As a result there can not be two individuals even of the same species, though they be brothers, who are identical. This has been disclosed by simple observation, for man, for animals and for plants. Pasteur showed that it is the same for bacteria. In addition to the general characters belonging to the species each bacterium, even in the same culture, presents special characteristics. Let us note in passing that in this case, this difference in the properties of bacteria within a single culture—that is, where all of the individuals find themselves in the same environmental conditions—can only be explained as the result of an inequal transmission of different characteristics. This difference among the bacteria of a single culture is particularly outspoken as regards their virulence.

Just as there are no two men who are absolutely identical, so there are no two plague bacilli which can be absolutely the same. It does not suffice to place into contact a man and a plague bacillus that there may be infection, it is essential that the man and the bacillus each possess particular attributes. The problem is the same, whatever may be the bacterium which attacks, whatever may be the animal attacked.

From the point of view of infection, the sum total of the characteristics of the parasite is termed virulence, the total of those with which the host is endowed is called susceptibility.

VIRULENCE OF THE BACTERIUM

To Pasteur we owe our idea of the variability of the bacterium. He showed that it is possible to vary the virulence of a bacterium by subjecting it to a series of passages through the body of an animal, in this way increasing the virulence, and he showed likewise, that the virulence of a bacterium can be attenuated by subjecting the bacterium to certain conditions in an artificial medium or to an abnormal temperature, or by passing it through the bodies of animals. The possibility of attenuating the virulence of certain micro-organisms is the basis of the Pasteurian method of vaccination with attenuated viruses.

Anti-anthrax Vaccination

B. anthracis is a spore-bearing organism, and this perpetuation by spores maintains the species fixed. Pasteur, Chamberland and Roux were the first to try to overcome this difficulty. They observed that in cultivating the bacillus at 42.5°C. it lost its power of producing spores, and at the same time it became attenuated, the degree of attenuation being related to the length of time the bacterium was heated at this temperature. Through heating the virulence is lost, first for cattle, then for the sheep, then for the rabbit, next for the guinea-pig, and finally for the mouse, the most susceptible of animals. Attenuation to this last degree is obtained after heating from twelve to twenty days. If the heating is stopped while the bacterium still presents the power of killing the new-born mouse the virulence can be re-established. It is only necessary to inoculate the blood of the new-born mouse into a young mouse, which will succumb, next the blood of the young mouse is injected into an adult mouse, then a guinea-pig receives the blood of the mouse, then a rabbit receives that of the guinea-pig, a sheep is next injected, and finally a cow. Thus, the virulence can be increased to a value such as it had before the attenuation. If, at a given time, the attenuating action of temperature is interrupted, spores form, and they fix the properties of the bacterium at the same time. A strain of bacilli is thus obtained which conserves, within certain limits, the newly acquired characters.

To obtain an effective vaccination it is necessary to inject the animal that one wishes to immunize with a culture from a strain of the bacillus having a virulence sufficiently attenuated not to cause a severe infection, but yet sufficiently high to cause the body to react. Since such a reaction can not be obtained with a single injection without danger, Pasteur and his collaborators had the idea of inducing the reaction at two times; a first injection of a culture sufficiently virulent to kill an adult mouse but which spared the guinea-pig and which provoked in the sheep or cow only a slight transitory malaise, but which conferred a resistance sufficient to enable the animal to support some twelve days later a second injection of a more virulent culture, one which killed

the guinea-pig but spared the rabbit.   Some twelve to fifteen days after this last injection the animal enjoys a solid immunity which persists for one to two years.

The virulence of the anthrax bacillus may be attenuated by means other than heating.   It has since been recognized that it is possible to obtain the same result by cultivation in media containing certain antiseptics (Chamberland and Roux), by subjecting the culture to the action of oxygen under pressure (Chauveau), or even to solar rays (Arloing).

THE CAUSES OF VARIATION IN VIRULENCE

There are, then, two methods of attenuation.   In the one the lowering of virulence is obtained in vitro by the action of chemical or physical agents, the other operates in vivo, but in both cases there is an adaptation of the bacterium to the new conditions of its existence.

Attenuation or exaltation in vivo results from the faculty which the bacterium acquires of developing at the expense of the substance of an individual, or secreting ferments which permit it to decompose and utilize this substance, but the acquisition of this property against a representative of a given species may gradually weaken the power of utilizing the substance of an individual of another species.   Experiment shows, in fact, that this is the case.   A microorganism adapts its production of ferments to the food which it finds available.   Yeast in a medium containing maltose produces maltase, a ferment which it does not form in a medium containing saccharose (Bourquelot).   *Aspergillus niger* can utilize tannin, thanks to a tannase, but this is produced only in the presence of tannin (Pottevin).   If they were pathogenic bacteria we would say that the virulence of the yeast is enhanced for maltose, that of the Aspergillus, for tannin.

Such direct experiments are much more difficult to accomplish with pathogenic bacteria, since these do not act on well-defined and titrable chemical substances, such as maltose or tannin, but upon complex substances.   Despite all this, the following experiments show that the mechanism is the same.

The bovine species alone is naturally susceptible to pleuropneumonia.   By cultivating the Asterococcus, the virus of this

disease, in goat serum, Dujardin-Baumetz has succeeded in rendering it pathogenic for the goat. Cultivated in horse serum it acquires virulence for the horse, and in an inverse proportion it loses in virulence for the bovine species. But re-cultivation in beef serum causes it to regain its virulence for bovines.

But a bacterium may acquire virulence for an animal by means other than cultivation in serum, a fresh organic product.

During an investigation of hemorrhagic septicemia of bovines d'Herelle and Le Louët noted that the pathogenic bacterium gradually lost its virulence for the cow if it was subjected to passage through the rabbit. After a certain number of these passages, it is possible to inject without danger a cow with 1 cc. of a culture obtained by seeding bouillon with the blood of the last rabbit of the series, when a dose 5000 times weaker of a culture of this bacterium, which has not passed through the rabbit, causes the death of the cow in less than twenty-four hours. The attenuation so produced is like that of the rabies virus; the phenomenon is not new. But here is an unexpected observation. With inoculation of one portion of bouillon prepared from beef meat, and another part prepared from rabbit meat, and cultivation for eight days, cultures are secured which upon test show that the injection of a cow with 1 cc. of the culture in rabbit meat bouillon is inoffensive and vaccinates the animal, while a dose 100 times weaker of the beef bouillon culture kills the animal in twenty-four hours. What is the explanation? The bacterium by passages through rabbits is adapted to the rabbit medium, it accustoms itself to develop at the expense of rabbit substance, and it becomes gradually unadapted to the conditions of the beef medium. Injected into a cow, the body of this animal reacts and dominates the situation before the microörganism has had time to habituate itself. The bacterium conserves its characteristics acquired in the rabbit medium if cultivated in the rabbit bouillon, but on the contrary, if it is cultivated in beef bouillon these characters become effaced little by little, and it acquires without hindrance an adaptation for the beef medium, for the bouillon does not defend itself. Injected into the body of the cow the bacterium is ready to develop immediately.

It is certain that the biological conditions of culture media exercise a considerable influence on the properties of bacteria which are cultivated therein, but up to the present time these conditions have been completely neglected. Through the influence of the work of Sörensen, innumerable investigations have been made during the last few years upon the chemical conditions of culture media. These studies have yielded interesting results, but they have nevertheless obscured the point of view that the biologic conditions of a medium exercise an influence still more considerable. One might affirm in principle, that the chemical conditions of a medium are preponderant in that which concerns the results of ordinary chemical investigations; the biological conditions in that which deals with the biologic investigations. For example, for the bacterium of hemorrhagic septicemia, the degree of alkalinity of the medium, within the limits compatible with the normal life of the bacterium, is of little importance as to the degree of virulence for any given species of animal. But the kind of meat used in the preparation of the medium is very important; it modifies the properties of the bacterium for any given animal species.

It would have been supposed that immediately after the discovery by Pasteur of the procedure for the attenuation of bacteria we would have been in possession of a general method which would allow us to vaccinate against all infectious diseases. Unfortunately this has not been the case. The diverse bacterial species do not all comport themselves in the same manner, but this is not the great difficulty in the application of the method of vaccination by attenuated viruses. Vaccination against anthrax involves the death of certain very susceptible individuals. As concerns animals this is not so serious; it is simply an economic question. It is advantageous to vaccinate against anthrax, for although the loss from the vaccination may reach 1 per cent of the herd or the flock, without it 25 per cent will die of the disease. It is clear, however, that when the problem is applied to man, it assumes an entirely different aspect.

However this may be, it can not be said that we will not return to the Pasteurian method of vaccination by attenuated viruses when a more profound knowledge of bacterial biology will have

permitted us to perfect it. It is, at the present moment, the sole method of vaccination of which the efficacy is not subject to discussion.

The conditions of infection are, then, extremely complex, since on one hand, each bacterial species possesses certain characteristics common to all of the bacteria which compose it, and on the other hand each animal species possesses on its side a certain number of general characters which cause, inherently, such a bacterial species to be pathogenic for that particular animal species. It is essential not to forget that the individual bacterium which may be implanted in the body of an animal possesses its own peculiar characters which may exercise a preponderating influence on the possibility of infection in each case which may be presented. It is not the general characters of the two species involved, but indeed the particular characters, inherited or acquired, of the two individuals in the struggle, which determines whether the attack will prevail over the defense, or whether the defense will be adequate.

We have seen the nature of these particular characters in the bacterium, let us pass to a consideration of the host.

SUSCEPTIBILITY

All of the higher vertebrates are susceptible to tuberculosis, and although only a few species are subject to the natural disease, in them a natural infection is produced only under particular conditions.

In man there is a relation between the degree of civilization and the mortality from tuberculosis. The reason for this fact is that the contagion has become exalted because of the congestion of the people and because of the confined life. Similar conditions of infection are to be observed for the bovine species. In countries where the animals live at liberty in large pastures the morbidity from tuberculosis is extremely low, while it may reach as high as 80 per cent, and more, among animals which are confined in stables. But this is not all. The guinea-pig is extremely sensitive to tuberculosis, even spontaneously, as may be shown by causing it to sojourn in the room of a hospital for the tuberculous. Nevertheless it is exceptional to find a case of natural infection under the conditions of the normal existence of this animal. Calmette seems to have found the reason for these facts.

Contrary to the opinion very widely held the contagion does not enter, usually, by the respiratory passages, but by the digestive route through the intermediary of the foods soiled by the excretions of patients affected with tuberculosis, excretions which contain an abundance of pathogenic bacilli. And, the more pasty the excreta the greater the danger of contamination. If the guinea-pig escapes natural infection, while man, and cattle especially, are severely infected, it is due in part at least to the consistency of the fecal material.

It appears that for an infection to occur, it is not only necessary that a species be sensitive; there are still other conditions which must be realized.

Another example—the buffalo is the beast of burden which replaces cattle in the hot and wet regions of the old world. Here, the buffaloes are decimated by a bacterial disease, hemorrhagic septicemia or barbone, which is, without doubt, the most highly fatal of all known diseases. In this disease morbidity and mortality are equivalent terms; with only very rare exceptions all affected buffaloes die. But the cow is likewise very susceptible, nevertheless statistics show that the mortality from barbone is very low in these animals. The reason is simple. The bacterium of barbone remains alive for a very long time in the mud of the water-holes frequented by the buffalo, which is a semi-aquatic animal, and where the cows, which prefer the dry regions, never enter. In dry regions barbone does not rage, and it is hardly possible to find there an isolated accidental case. It is only necessary to cause a herd of cattle to dwell in a swampy region to see the epizootic manifest itself with a mortality as high as that present among the buffaloes.

In countries where yellow fever exists in endemic form the natives are free, only the stranger is affected. Statistics suggest that the native is non-susceptible to yellow fever, and this has been the general belief for the past thirty years. Nothing is less true, for if the native is removed for several years from the endemic focus, he is affected like the stranger when he returns. The native of the same race living outside of the coastal zone, that is, in a non-infected zone, is as sensitive as is the stranger when he comes to the coast. In Central America some points

on the coast even are not infected, the littoral of Guatemala in particular, but, when the virus is imported by a patient the epidemic which results is strikingly fatal. I assisted in 1906 at an epidemic of this nature, the only one which has occurred there during the last century, and in certain regions the mortality reached as high as a third of the population.

The native is, then, very susceptible to yellow fever in the regions usually non-infected; he is refractory in contaminated regions. Simond, Marchoux and Salimbeni have given the reason. Yellow fever, a disease often fatal for the adult is very benign for the infant, in which it assumes the form of a simple malaise, usually passing unperceived. This benign attack immunizes, and the immunity is reinforced and maintained by the bites of the infecting mosquito, the inoculator of the virus, bites received from time to time as the individual sojourns in the infested region.

A very interesting case is that of malaria. The following fact has often been observed and has received abundant discussion. In certain districts malaria has disappeared for a period of a few years while the disease persisted throughout the surrounding territory, and at such times it has been impossible to observe any diminution in the number of Anophelines, the agents of transmission. There must assuredly have occurred in these regions an event, unnoticed, which has led to a change in the conditions, either of the mosquito or of man.

Roubaud has attempted to explain this disappearance of malarial fever in the following manner. Anophelines do not bite man exclusively. They feed upon a number of animals, particularly upon the domesticated animals, especially cattle and swine. There may even be a predilection for these last. If stabled animals are to be found in proximity to human habitations the animals are bitten in preference to man. Thus they will not become infected nor will they transmit an organism of which they are not carriers. The extinction of malaria is explained, according to this theory, by an increase in the number of domestic animals found in the neighborhood of human dwellings.

I do not hesitate to state that this explanation fails to satisfy me, for indeed it can not be applied in all cases and, furthermore,

it does not take into account the fact that in almost all of the regions where a local disappearance of malaria occurs the animals do not live in stables during the summer and autumn months, which are precisely the months when the disease prevails.

With reference to this very important question of the prevalence of Anophelines without a corresponding prevalence of malaria Grassi has recorded the following. In certain districts of Tuscany where malaria had been absent for some years the disease reappeared upon the return from the Balkans of some of the troops who contracted the disease during the campaign. The cases to develop were somewhat peculiar in that the disease was of a benign character; in some cases, even in the absence of specific quinine therapy, recovery was spontaneous. Grassi concluded that the mild character of the disease could not be ascribed to the environmental conditions, for the soldiers who had contracted the disease in the Balkans were severely infected and proved to be relatively resistant to quinine therapy. Furthermore, the factor associated with the attenuation of the disease bore no relation to any special resistance of the inhabitants, since the soldiers themselves were natives of Tuscany. The sole explanation offered by Grassi is that in regions where malaria has disappeared spontaneously the Anophelines are still able to propagate the malarial parasites, but that the latter undergo within the mosquito an attenuation in virulence.

After having eliminated the domestic animal factor, which, as he demonstrated could not be invoked as an explanation, Grassi ascribed this attenuating factor to the fact that the mosquito larvae had developed in a pure water. "Brackish water" he states "increases the virulence of the malarial parasites." Such an explanation would be admissible if the parasite was transmitted by the egg of an infected insect to the progeny, as is the case for the Piroplasma of Texas fever in cattle, where the transmission is from the maternal tick to the daughter ticks. But such a mechanism can not be the case here. How can the place of the development of the larvae operate as an attenuation factor upon a parasite which the mature insect, derived from this larva, does not ingest until a long time afterward?

I have had occasion to study this question of anophelinism without paludism and I have arrived at conclusions of a quite different nature. These conclusions fit in perfectly with the facts observed by Grassi.

The greater portion of the Argentine Republic is completely free of malaria, in spite of the fact that the Anopheline carriers abound in these regions where very frequently are to be found human carriers of the plasmodia, who have contracted the disease in the malarial districts (Provinces of Salta, Tucuman, and Jujuy). On the other hand, the beasts of Argentina are never stabled and are never found in the immediate vicinity of habitations. Moreover, I have established personally that Anophelines are at times very abundant and bite man with fury. In the Republic of Argentina and in the neighboring and equally uninfected districts, such as Paraguay, the domestic animal factor can not be invoked.

During the course of many journeys to the interior of Argentina I attempted to ascertain the causes of this strange immunity, and I have had in this the aid of an agricultural inspector of the country, Mr. Tribodi, who accompanied me and who is very familiar with the flora of these regions. We have seen that in all of the free regions there is a wild plant, probably imported from Europe at the time of the first Spanish occupation and now abundantly disseminated, called by the natives "trebol de olor" (scented clover), belonging to the genus Melilotus, probably a local variety of *Melilotus altissima*. Flowering takes place during the critical period of malaria, that is, from the beginning of summer to the end of autumn. The highly scented blossoms are continually frequented by insects of many kinds, and particularly by Anophelines, which feed upon the juice which, like that of all plants of the genus, contains a glucoside, coumarine. This plant is not present in malarial districts.

It is known that Anophelines, males and females, can feed upon vegetable juices. Only the fertilized female requires the ingestion of blood to insure the development of the eggs. May coumarine play a rôle in the insects comparable to that which quinine plays in man?

Upon my return to Paris I discussed this observation with the late Doctor Laveran, who considered it as possible. Despite the

fact that he had not seen the facts themselves the evidence in favor of this hypothesis could not be dismissed off-hand.

Recently, while living in Holland, I have sought the cause of the disappearance of malaria, as has been noted for about twenty years, from certain islands of Zealand and from the northern provinces of Holland. In these two regions the disappearance of malaria coincided with the accidental introduction of plants of the genus Melilotus, the seeds of which were imported from France with forage seed, lupine, whose culture in Holland commenced at that time. Malaria has, on the contrary, continued to ravage certain of the Zealand islands where lupine has not been cultivated, and where consequently, the Melilotus has not been introduced. These findings lead me now to publish these observations, and it will be, indeed, very easy to see if they are correct, since it can be proved by simply introducing these plants into a malarial region. Apparently the trebol de olor of the Argentine is the most effective species.

However that may be, whether it be the condition of the domestic animal population, whether it be the Melilotus, it is clear that some factors are able to determine the absence of contagion in so far as malaria is concerned.

Examples of comparable facts might be multiplied. It is, then, well to reserve a conclusion regarding the non-susceptibility of a given species, if such a conclusion is based upon the simple fact that this species is never attacked under the natural conditions of its existence.

But there is yet more. A pathogenic bacterium establishes itself in a susceptible individual; does it invade the body? Not always. We harbor pathogenic bacteria at all times without having our well-being manifestly affected. We are "healthy carriers." The pneumococcus, agent of lobar pneumonia, is a normal inhabitant of the throat of man. There also is frequently to be found the diphtheria bacillus, and even the meningococcus may occur in healthy persons.

One might try to explain these facts by admitting that these persons are refractory to the disease of which they harbor the bacterium, but this is not true, for it has been possible to demonstrate in many cases that after some days or some weeks the carrier has contracted the disease of which he harbored the germ.

It is not sufficient, then, that a susceptible individual find himself in contact with the bacterium for the latter to be able to develop; it is necessary that he be receptive at the moment of contact. All that contributes to weaken the body diminishes the resistance and favors the infection, which, without such a weakening, would often not be able to take place. Among the causes favoring infection intercurrent infection should be considered as most important. Influenza is a disease relatively benign by itself, but it favors pneumococcal infection which is far more serious. A catarrhal diarrhea which is not serious, provoked by cold or by the ingestion of undigestible foodstuffs, favors the development of serious intestinal diseases—cholera, typhoid fever, and dysentery. In all tropical couutries where cholera is endemic a very pronounced recrudescence of cholera occurs at the beginning of the mango season, since the natives often consume these fruits in excess and when unripe.

Cold, extreme heat, excesses of all kinds—fatigue, deprivation, alcoholism—diminish the resistance and favor infection. With regard to deprivation, the observations of Lubarsky are interesting. During the course of the war the Russian prisoners in Germany, reduced to a single German food ration, were found in a state of chronic famine. Tuberculosis among them became very frequent, assuming the grave forms with rapid progression, such as miliary tuberculosis, tubercular meningitis, exudative pleurisy, and serous peritonitis.

Age is also of considerable importance with reference to infectious disease. We have seen the relation of the severity of yellow fever to the age of the patient. The infant is rarely affected with diphtheria. Beyond the age of forty man is in general protected from typhoid fever. We will later see the reason.

The communal life of a large number of individuals, especially if they are individuals of the same age, that is, when they possess an ensemble of characters of the same general nature, as is the case with men living in barracks, strongly favors infection.

As is seen, the question of susceptibility is very complicated. There is no general law. Too many factors, in their essence variables, enter into consideration to allow any idea of determinism as regards contagion.

UNDETERMINED CONDITIONS

After all that has been said it might seem that we know suffi-
ciently well the conditions of infection, that the only thing remain-
ing is the elucidation of these questions in detail. This is, more-
over, the impression that one experiences after reading the texts on
immunology. All appears clear. But in reality it is quite other-
wise. Although for some few infectious bacterial diseases (I do
not speak now of protozoan diseases, for which the principal
facts concerning the mode of contagion are unknown), such as
tuberculosis, glanders, plague, and tetanus, it may seem that we
know well enough how the disease is contracted, for the others
we are as yet in the dark, and this ignorance is of the highest
order as we will see. A few examples of our inadequate knowl-
edge will be mentioned, and I will select these examples from
among the diseases that I have particularly studied.

There is in tropical countries a disease of the buffalo, barbone,
which belongs among the group of the hemorrhagic septicemias.
The blood, the organs, and the feces of dead animals contain an
enormous number of an ovoid bacterium, a pasteurella, which
grows readily upon the usual culture media employed in bac-
teriology. The virulence of the cultures is such that the
injection of a buffalo with 0.0005 cc. causes, without excep-
tion, an infection fatal in less than thirty-six hours. The
experimental infection and the natural process appear to be
identical in duration, in symptoms and in lesions, even to the
smallest details. It is thus certain, in so far as it is humanly
possible to be certain, that the pasteurella is the true agent of the
disease. The three postulates of Koch are strictly complied
with. How is infection accomplished? Very simply, it appears.
The bacillus can be isolated from the mud of the swamps fre-
quented by the buffaloes. A diseased animal contaminates the
mud with his excreta containing the pathogenic bacterium.
Healthy animals ingest these bacteria, especially in licking them-
selves. The pasteurella passes most often into the circulation
through the region of the throat, but sometimes through the
intestinal wall, as autopsy shows very clearly.

Here, one might say, is the disease par excellence for study,
for it is possible to reproduce experimentally, with exactness,

the natural infection, a condition without which experimental investigations are not free of error. I had thought this. But let us go on.

Barbone is without doubt the most highly contagious disease. I was present, in Indo-China in 1920, at an epizoötic which ravaged an extent of territory of some thousands of square miles in a very swampy and thinly populated district. The villages are some 10 to 12 miles apart, separated by almost impassable marshes, and the inhabitants communicate with one another only by boat upon the innumerable rivers which traverse the country. The buffaloes of one village have therefore no contact with those of the neighboring villages. It would appear that all of the needful conditions were here present to avoid contagion.

But, in the epizoötic which I observed, in less than three weeks, the disease was disseminated throughout all of this territory. Not a single village was spared, and the epizoötic persisted for about five weeks, during which rather more than half of the buffaloes of the region died—about twenty thousand. The authorities of each village maintained a registry of these animals, for the taxation of the country is based upon the head of buffaloes, and every time that an animal dies its owner hastens to make it known. Thus, it was only necessary to consult the register to determine the striking spread of the epizootic. The disease broke out simultaneously in villages some 20 to 30 miles apart, and in each village in several stables at the same time. How could this fact be reconciled with the possibility of contamination with the pasteurella contained in the mud of the marshes? Fact indeed forces us to think that the disease is caused by the pasteurella, *but that it is not that which is the cause of the infection*. This seems a strange contradiction, but the following seems to offer some confirmation.

During the course of the study of this disease, I had at Saïgon, that is, within an area free of barbone at a distance of more than 300 miles from the infected region, a stable where I always kept about 30 animals; the experiments being conducted upon a total of 150 buffaloes and bulls. The control animals, inoculated with the pasteurella, contracted the disease and died. They had always been, intentionally, scattered in the midst of normal healthy

animals, and the opportunities for contact were incessant.   Indeed, they were favored in that both the normal and the sick animals were made to drink from the same pail, and eat from the same rack, and, in addition, a healthy animal was always attached to the same ring as an infected one and remained there after the death of its companion.   Thus, although the chances for contamination were multiplied as far as possible, nevertheless not a single case of transmission was observed.

One conclusion seems unavoidable, namely, that we are ignorant as to how, in nature, the contagion of barbone is accomplished. I have reason to say, I think, that the unknown factor which remains to be explained is of the first order.

Another fact, among others which might be cited.   Recently there have appeared in America several memoirs upon epizootics. The authors have studied from this point of view the disease of mice caused by *B. typhi murium* of Loeffler, or related organisms. This bacillus is so pathogenic for this animal that the ingestion of 0.000001 cc., and even less, of culture invariably causes an infection which is fatal within three to four days.

In 1912 I was interested in this disease, with the hope of finding a test disease identical with natural disease.   I attempted to cause experimental epizootics by placing an infected mouse in cages containing some 20 healthy mice.   These tests were made upon about 200 mice.   But, I was never able to observe a case of contagion, my results being thus different from those of the authors which have been mentioned.   The reason lies in this difference.   I naturally took pains to remove from the cage the moribund mouse, just before death, for the mice devour the bodies of their congeners.   And it is evident that although a mouse which eats the organs swarming with bacteria will contract the disease, one can not speak of contagion in this case.[1]   It is clear from reading the papers cited, that this is the reason for the results obtained by the American authors, for they observe that

[1] The rats eat the cadavers principally for the water contained in the tissues.   This is astonishing to bacteriologists perhaps, since the mouse is considered the animal which, of all perhaps, has the least need for water. But having water freely available a normal mouse will drink from 3 to 6 cc. per day.   For the nursing mouse the daily amount may be as high as 20 cc.—more than its weight.

the mice practise cannibalism and that this explains in part, they say, the results of their experiments. It is not "in part," but "entirely" which they should have said.

Having the responsibility, in 1918, of organizing a campaign against field-mice which had multiplied during the war in the zone of the armies to such a point that in certain regions all cultivation had become impossible, I had in my laboratory some thousands of field-mice which had been sent me for use in increasing the virulence of a pathogenic bacillus.[2] I had the opportunity to repeat on a large scale the experiments made upon white mice, and this the more readily since these animals more rarely practise cannibalism if they are provided with an abundant water supply. The result was the same. It was absolutely impossible to obtain contagion. I have investigated whether the same condition was to be found in the fields that I had infected with grain contaminated with the cultures. This demonstrated that epizootics never occur, as was readily determined, for although one found after some days a number of dead mice throughout the region where the bait had been spread, at fifty meters from there not a single one was to be found. The virus works like a poison; only those mice who eat it contract the disease and die.

Nevertheless, we know that in nature, when an epizootic breaks out among the field-mice the contagion is accomplished with great rapidity. But we do not know how this contagion is effected.

Such examples might be multiplied, and we will see others when we come to try to uncover the unknown cause.

In any case, with reference to the point in which we are at the moment interested, it is possible to say this, recognizing that Koch has advanced the following as the three basic points establishing the rôle played by a bacterium in a disease—(1) the suspected bacterium should be found without exception in all diseased organisms, (2) this bacterium should be isolated in pure culture, and (3) the administration of these pure cultures should

[2] In order that the infestations may give a good result it is necessary to seed the bouillon (or rather the medium, very economically prepared with 5 grams of salt and 25 grams of bran per liter) with *B. typhi murium* in a very virulent state, that is to say, isolated from the heart blood of an infected field-mouse.

cause the disease—that to these conditions it is essential to add a fourth, namely, that the experimental disease should be as contagious as is the natural disease. This last condition is the principal one as regards experimental disease, for it, and it alone, is sufficient to demonstrate the rôle of the supposed agent. When this is fulfilled the three postulates of Koch are unnecessary; when it is not satisfied the three cardinal points can only lead to error. They may incriminate as morbid agents organisms which are merely associated forms. This is a question to which we will return.

There is then, a group of epidemic diseases for which the cause of the contagion remains unknown. To what group do these diseases belong?

In principle, with the exception of a few diseases caused by toxic agents, such as tetanus and botulism, all infectious diseases are contagious, but the contagious character is extremely variable. An observation which has certainly been made by every physician, but upon which no one seems to have placed sufficient emphasis is that there are two forms of contagion and that it is in truth not logical to designate the two forms by the same name. No one would attempt to prove the identity of the contagion in tuberculosis, for example, with that of plague, of cholera, or of measles. Certain diseases present features which accord with the circumstances of the one or the other mode of contagion, such as bronchopneumonia or typhoid fever. One should speak of those diseases which fall in the class with tuberculous as transmissible infectious diseases, reserving for the others the term contagious.

We know quite well the general modes of transmission of the diseases simply transmissible, and it is to those in particular that what has been said in the chapter on the conditions of infection should be applied. In so far as they are concerned the experimental disease is as little contagious as is the natural disease. As regards the diseases really contagious, that is to say, those which spread rapidly and to a distance, they may be divided into three groups:

1. The diseases caused by invisible agents. These are the most contagious. Consideration of them is reserved for Part Four of this text. Only let us remark here that in their case

the experimental disease presents the contagious characters of the natural disease.

2. The diseases transmitted through the intervention of insects, such as plague, malaria, sleeping-sickness. With these the epidemic character is explained and it only remains to elucidate some of the details.

3. The diseases, often very contagious, *apparently* caused solely by definite bacteria, such as cholera, typhoid fever, and the pasteurelloses of animals. Here the experimental disease is not contagious, and it is in this respect that the cause remains unknown.

CHAPTER III

The Passive Defense Against Bacteria

THE DEFENSIVE PROCESSES

To the attack of bacteria the body opposes varied means of defense, which may be divided into two classes presenting very different interests. The passive means of defense, which are but little subject to variation and which function indifferently in the defense against the attack of any species of bacteria, are derived from the structure of the being which they defend. The active means of defense, which the individual possesses naturally, or acquires by adaptation, are specific, that is to say, they only operate against the bacterial species which has brought about this adaptation.

These two modes of defense were the only known mechanisms prior to the investigations of d'Herelle. This author has shown that the phenomena of defense are infinitely more complicated than had been supposed heretofore. The game is not played by two organisms, the bacterium which attacks and the animal which defends, for there is a third being which intervenes, the ultramicrobic parasite of the bacteria, the bacteriophage. There is a superposition of parasitism, the ultramicrobe developing at the expense of the bacterium which, in turn, tries to develop at the expense of the animal.

May the bacteriophage be considered as designed to effect a defensive function? By no means, although animals profit by the parasitism exercised by this bacteriophage for the bacteria. But if, of all living beings, the bacteria alone escaped parasitism, the result would be simply this, either evolution would have followed another course and immunity would have been assured by other means, or evolution would not have passed the stage of the unicellular organism. It is simply a question of the possibility of adaptation. The bacteriophage does not exist for the purpose of defending animals against the attack of bacteria, it exists because

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in the course of time an ultramicrobe has parasitized the bacteria after the same manner that the bacteria have parasitized animals.

THE PERIPHERAL DEFENSE

First, let us consider briefly the non-specific means of passive defense which the body opposes to bacterial invasion.

The skin forms a barrier interposed between the environment and the tissues. The epidermis consists of several layers of cells. Those of the upper strata have undergone gradual transformations of composition and of form; they have flattened out and have changed to less vulnerable substances in such a manner that the superficial layers are formed of cornified scales, overlying one another like the slates of a roof. The skin constitutes an efficient barrier to the penetration of bacteria, for, by their composition these superficial layers resist the attack of the bacterial ferments and by their arrangement they do not offer openings adequate for the bacteria to enter. Let us add that these superficial layers, thrust upward by the cells which are underneath and which form continually, exfoliate, and this brings about the removal of the bacteria which have been able, despite all, to infiltrate between the horny plates. In certain cases the irritation produced by the implantation of a cutaneous parasite provokes an exaggeration of the process of elimination, and there then occurs an intense desquamation, as represented by the skin affections.

The cutaneous protection ceases if the arrangement or the texture is modified even so little as by a scratch or even a less marked traumatism, by the bite of an insect for example. The plague bacillus is ordinarily transmitted by the flea, but it seems that in this case, indeed, if not habitually, it is not inoculated by the insect but is found in the particle of excrement deposited at the moment of sucking the blood. The insect having departed, the bacillus is introduced into the minute cutaneous lesion by the friction of the clothing or by scratching.[1]

[1] Contamination by the flea is certain, but is this the only mode of contagion? This is hardly probable. It is known that plague in the rat may assume a chronic form, and that in this case, the bacilli are to be found in the excreta of the animal. The contamination of man by the ingestion of foodstuffs soiled by such excreta must certainly be considered a possibility. It may even be that in certain cases these processes of infection intervene more frequently than the first.

Sometimes a bacterium succeeds in forcing the barrier of the epidermis, producing in this way, for example, a lupus or cutaneous tuberculosis. The connective tissue of the dermis reacts, then, under the influence of the irritation produced by the secretions of the parasite, it thickens, forming veritable fibrous walls which localize the infection. This reaction constitutes a powerful means of defense. Thanks to this, cutaneous tuberculosis propagates itself only slowly and without the tendency to invade the internal organs.

THE DEFENSE OF THE MUCOUS MEMBRANES

The mucous membranes, nasal, respiratory, and ocular, by virtue of their anatomical structure would constitute a barrier less difficult for the bacteria to penetrate than the skin, if the passive obstacle constituted by the juxtaposition of unassailable elements were not replaced by the secretions.

With regard to the eye, the bacteria which are deposited on the surface of the conjuctiva are carried by the lachrymal secretions to the nasal cavities. In spite of this, the conjunctiva is certainly one of the weak points of the organism. For example, Calmette has demonstrated that it is sufficient to instill a drop of a culture of tubercle bacilli into the eye of a guinea-pig to produce a rapidly developing tuberculosis. The tubercle bacillus is not, however, the only one which is able to penetrate by this route; it is the same with the virus of rabies, the plague bacillus, etc.

The defense of the respiratory mucosa is more efficacious, by virtue of secretion of mucus and by the functioning of the lining cells. Lermoyez and Wurtz have attributed to these secretions a bactericidal action but their opinion has been vigorously opposed. We have seen that the mucin tends to hinder the action of the ferments; it seems, then, that without being endowed with a true bactericidal action the mucus must at least impede the activity of the products of secretion of the bacteria, and by this embarrass their development.

Bacteria entangled in the secretions of the mucous membranes are next expelled by the action of the ciliated epithelium, which propels the particles of mucus from the depths to the exterior. The same conditions of resistance and of mechanical elimination

are found as far as the pulmonary alveoli. Through the whole extent of the trachea, but principally in the alveoli, there is, in addition, another process of defense provided by the "dust cells," cells given this name because they often contain grayish particles. These are the large mononuclear leucocytes which engulf all foreign particles,—dusts, spores of molds, and the bacteria present in the inspired air. It is needless to consider these further at the moment, for we will have to return to this at length in dealing with phagocytosis.

THE DEFENSE OF THE DIGESTIVE TRACT

The digestive tract is peopled with bacteria from the first few hours after birth. The ingestion of bacteria is indeed constant. They are found in suspension in the air, lodging on all foods, contained in all liquids. Not only the saprophytic bacteria, but also the pathogenic forms are distributed in the external environment, disseminated there most frequently in the excreta of patients, spread about with the expectorations, or contained in the purulent discharges of wounds.

Aside from rare exceptions infection is effected by way of the digestive tract. It is thus that the meningococcus, the agent of cerebrospinal meningitis, penetrates the body at the level of the tonsils, and the bacteria of the hemorrhagic septicemias gain entrance most often at the same point. More rarely, the latter enter through the intestinal mucosa, as is also the case for the anthrax bacillus. It was believed for a long time that infection in pulmonary tuberculosis was always effected by the aerogenous route, but this is not true, the penetration is usually through the digestive tract.

The tonsillar ring forms one of the weak points of the body. The mucous layer is continually traversed by the migratory cells, the leucocytes, which engulf the foreign particles, bacteria in particular, which are deposited at this level. If it is a saprophytic bacterium the leucocyte digests it, in the contrary case the microorganism resists. Hence the rôle of phagocytosis may perchance be disastrous, for the leucocyte then performs the office of a conveyor, insinuating itself between the cells of the mucosa it, together with its contents, re-enters the circulation. There, the

bacterium, still enclosed but perfectly alive, becomes liberated and begins to multiply. Calmette in particular, has demonstrated the harmful rôle of leucocytes in so far as tuberculous infection is concerned.

In the digestive tract, as in the respiratory tract, there occur mechanical phenomena of expulsion which contribute to the elimination of bacteria, by the throwing off of saliva from the upper passages and the excretion of fecal matter from the intestine.

The stomach, by reason of the secretion of hydrochloric acid, certainly forms a barrier for certain very fragile forms, but this barrier is readily penetrated by the majority of species. As for the ferments secreted there, they are without action on living bacteria.

The intestine, the large intestine in particular, forms the center of the defense of the body, and that by virtue of the presence of an ultramicrobe parasitic for the bacteria. We will consider the rôle of this ultramicrobe in a later chapter.

THE DEFENSE OF THE GENITO-URINARY ORGANS

The genito-urinary organs may also provide an avenue of access for bacteria to enter the body. From these organs the elimination of foreign particles is effected by a process analogous to that which occurs in the respiratory tract; mechanical elimination and vaginal desquamation. The vital competition which takes place between the saprophytic bacteria which reside there normally and the pathogenic bacteria which may be implanted there, must certainly be taken into account.

In so far as the genital organs of the female are concerned Gildemeister has demonstrated there the constant presence of the bacteriophage, and I have verified this for the female of the rabbit and the guinea-pig. This agent intervenes, then, there also, in preventing the invasion of this tract by the bacteria.

As for the urinary passages, the acid reaction of the urine is unfavorable to bacteria in general, which only vegetate normally in media slightly alkaline, or at most neutral. In certain pathological conditions, hypertrophy of the prostate, for example, the urine accumulates in the bladder and becomes alkaline, when it quickly becomes populated with diverse bacteria.

THE DEFENSE OF THE LIVER

Very often it happens that bacteria succeed in overcoming the obstacles opposed to their penetration, either by passing through by their own forces or by being introduced by a leucocyte. Entering into the circulating blood they encounter there still another barrier of greater efficiency.

The antitoxic action of the liver is considerable. It acts on numerous poisons and prevents the intoxication with which the body is continually menaced through poisons resulting from cellular metabolism and by toxins of bacterial origin, produced in the digestive tract and absorbed by the intestinal mucosa, or produced in the tissues themselves in the course of infectious disease. To this antitoxic action, which takes place through processes of a fermentative nature, must be added a mechanical purification.

The hepatic tissue is formed of epithelial cells whose walls are hollowed out into little grooves, and the union of such cells, with the corresponding grooves, forms fine canals, the origin of the biliary tubules. These cells are grouped in polygonal structures which constitute the hepatic lobules. The ultimate ramifications of the portal vein, which are to be found in the periphery of each lobule, provide the blood capillaries which traverse the lobule radially, and come to form by their reunion at the centre of the lobule a radicle, the source of the sub-hepatic veins. The liver presents a structure, then, which allows it to play the part of a filter, functioning for the purification of the blood.

Bacteria passing thus from the blood to the bile are evacuated into the intestine. The action is purely mechanical, for the bile does not possess any antiseptic properties. It is, on the contrary, an excellent culture medium for many bacteria. In "carriers" of intestinal pathogens it is in the contents of the gall bladder that the bacteria maintain themselves and multiply.

The proof of the elimination of pathogenic bacteria by the bile has been provided by Calmette with reference to the tubercle bacillus. This elimination is particularly marked in resistant individuals. The passage of bacteria through the liver is extremely rapid, as may be shown in guinea-pigs, where, with the

gall bladder brought to the skin by a fistula, bacteria injected into the circulation may be found in the bile within five minutes after the inoculation.

THE RÔLE OF FEVER

Before completing the discussion of the non-specific means of defense, that is to say, those defenses in which the mechanism is the same whatever may be the organism which threatens to penetrate the body, a word should be said with regard to fever.

Fever is a symptom common to all infectious diseases. Is it then, a defense, or a lesion? It is indeed difficult to say. Certainly, it is not in all cases consecutive to the secretion of a toxin by the invading bacteria, for the fever appears after the injection of killed bacteria, and even after the introduction of any albuminous substance whatever. We have seen that fever accompanies anaphylactic shock; it is only in the case of shock which is quickly fatal that it is lacking. Fever is provoked, then, not by the bacterium as such in the body, but by it as a protein substance. It would appear from this that fever must be a banal symptom, common to all infectious diseases, since all bacteria are composed of proteins.

In very rare instances, in cholera for example, there may be an absence of fever, and instead of providing a favorable prognosis it is the indication of a very severe condition. When the disease develops toward recovery fever appears.

It seems, then, that fever must be considered as a reaction tending to augment the intensity of the processes whereby foreign substances of albuminoid nature are eliminated from the body.

CHAPTER IV

INFECTION

THE PATHOGENIC BACTERIUM

Davaine was the first, who, in 1840, actually demonstrated a bacterium, observed in the blood of sick animals, as being the cause of a disease—anthrax. He was not able to furnish irrefutable proof because of an inadequate technic, and this demonstration of the bacterial agent in disease was reserved for Pasteur, which he provided in 1867 in connection with his investigations on a disease of silk worms, pébrine.

Since then investigations have been carried out on all sides, and the bacterial nature of a great many diseases has been recognized, so that it is considered proved that all infectious disease is incited by a germ which succeeds in penetrating the barriers encountered at the periphery of the body. This first obstacle overcome, does the infection proceed to a fatal termination whatever may be the invading germ? No, for the internal defenses begin to operate. But while the external defenses are not specific, that is to say, they function whatever the kind of bacterial antagonist, as regards the internal defenses it is precisely the variety of this bacterium which determines the intimate nature of the reaction which follows.

We have already seen that the introduction into an organism of colloidal protein substances, of bacterial or other origin, leads to modifications in the state of colloidal equilibrium of the blood, to physical modifications which have been considered as substances and to which have been given, incorrectly, the name of antibacterial antibodies. These modifications do not constitute, in any case, reactions against the bacteria. Quite the contrary, they constitute by themselves a pathological state, and are even able to involve the death of the individual in which they are produced.

On the other hand, the ferments of defense, which appear in the blood as a result of the introduction of foreign protein substances, are certainly the result of an effective reaction of defense which tends to degrade these proteins, and to change them to the form of crystalloids readily eliminated by the emunctories. In any case, the production of these defense ferments does not constitute a protection against the bacterium as a living being, but as a protein, and they can only be formed after the disintegration of the bacterial body within the organism, a disintegration which, itself, is effected as a result of processes of defense directed against the bacterium, the living being.

A bacterium does not act solely in a banal manner upon a living being within which it implants itself, because being formed of proteins, a bacterium, whatever it may be, is not an inert being. Like all living cells it produces ferments which endow it with the possibility of the assimilations and of the disassimilations which characterize life. It procures in the medium the substances which, degraded by the ferments of decomposition, provide energy, and it likewise procures there the material which, altered by the ferments of reconstruction, provides it with its own substance and permits it to multiply. But these diverse ferments are liberated in the medium, and the products of decomposition likewise diffuse. If this medium is a living being these are the very substances which disturb its colloidal equilibrium, and the disturbance provoked in this case is physical.

Certain of these substances elaborated by the bacteria possess, in addition, a special affinity for the cellular colloids of this living being, either because of the formation of a colloidal complex involving a dissemination or a flocculation, or because the bacterial product acts as a ferment causing the degradation of some of the fractions of the cell. Here a colloidal reaction results which tends to modify the cellular activity, the function, and the cell is no longer able to play its rôle in the economy of the organism. If this alteration involves a great number of cells, especially if they form a part of a group which assumes an important function in the organism, all of the harmonious equilibrium which normally exists in the organism is destroyed.

Natural disease and experimental infection

All bacteria which may succeed in penetrating into the organism provoke then, disturbances which are in proportion to the loss of equilibrium which their secretions produce. In natural processes we will see that in order to enjoy a pathogenic character the bacterium must in addition possess other properties such as will paralyse the means of defense. This is not true from the experimental point of view.

Let us take an example. The rabbit is completely refractory to human typhoid fever, the guinea-pig never contracts cholera, because these animals possess a means of defense which automatically eliminates all of the typhoid bacilli, or all of the cholera vibrios which may be implanted in them. There is not, therefore, a natural production in the bodies of these animals of the secretions of these organisms, since they are not able to develop there. The injection into these animals of a small quantity of a bacterium *naturally* inoffensive to them is followed by elimination and no disturbance results. If we inject them with a large quantity of a culture of this same organism, we introduce of necessity, the secretory products of the bacteria thrown out into the artificial media, and we then induce simply a disturbance of equilibrium. May we say that we have reproduced typhoid fever or cholera in this refractory animal? Not at all. It is a banal disturbance, resembling in no way the human infection, having no relation to it, and of such a nature that it may be produced by the injection of a large quantity of any bacterial culture whatsoever. The bodies of these refractory animals put into play means of defense tending to counterbalance the disturbed equilibrium produced, but these means of defense are not comparable to those which are operative in the natural infection of a susceptible animal, and this, because although in the susceptible animal these banal disturbances of equilibrium are produced, there are in addition other upsets, caused by the affinity of the products secreted by the bacterium for certain cells of the body. Here, there is no longer simply a loss of equilibrium, there is in addition a cellular disorganization.

In the refractory animal, the injection of a bacterial culture causes, then, a rupture in the equilibrium of the colloidal state of

the fluids. In the susceptible animal there is this same disturbed equilibrium and in addition a disorganization of one or several groups of cells, the sum total of these disturbances constituting the disease. In the susceptible animal the true disease is produced as the result of the introduction into the body of the pathogenic bacterium, whether it enter naturally or experimentally.

The experimental disturbance in a refractory animal and the natural or experimental disease in the susceptible animal, caused by the same bacterium, are fundamentally different. Nevertheless all of the phenomena of immunity have up to the present time been studied in the refractory animal and by means of the facts observed an attempt has been made to interpret the reactions of defense in the susceptible animal disregarding all of the phenomena which show clearly that this is a false method. Such a method can only lead to error, and that is what has happened. We have already seen it with regard to the antibodies called antibacterial, with regard to contagion, and we will find it again with regard to other conditions.

It has been more than twenty years since C. Fränkel wrote, concerning the experiments on the etiology of cholera, "In the actual state of our knowledge, we can not satisfy ourselves that the hecatomb of guinea-pigs and the enormous expense of effort and intelligence, expended up to the present time, have accomplished anything." Since then, the slaughter has continued, thousands of papers have appeared, and our knowedge of cholera is no more advanced than in the time of Fränkel, simply because the path blazed by the clear genius of Pasteur has been abandoned. Disease has been studied, the reactions of defense on the part of the body against the action of bacteria have been followed, not in the susceptible animal, but in the refractory animal. This statement does not apply solely to cholera, but to other diseases as well.

The characteristics of pathogenism

All bacteria capable of being implanted in a living being are liable to provoke there ruptures of the equilibrium, whether this action involves all of the colloids of the body, or whether to this general action is joined a specific action affecting one or several

definite groups of cells. Among these different groups of cells, there is one which, from the point of view of defense, interests us especially, namely, that constituted by the leucocytes of the blood which possess the property of engulfing foreign elements which penetrate into the tissues.

According as the products resulting from the vital activity of a bacterium of a given species exercise a toxic action or do not exercise it upon the leucocytes of individuals belonging to a determined species of animal, the bacterium is, or is not, pathogenic. In the latter case, the bacterium is phagocytized and destroyed upon its penetration into the body; it can not develop there, and as a result can not provoke any disturbance whatsoever. This species of animal is refractory to the disease caused by this bacterium; it enjoys a natural immunity. In the other case, phagocytosis is not operative, or if it does take place, the leucocytes are unable to digest the bacteria which may then grow within the interior of the leucocyte itself. The bacterium multiplies, the secretory products cause general disturbances by virtue of a loss in the equilibrium of the colloids of the body fluids, and upon these are superimposed special disturbances, more or less accentuated, resulting from the special affinity of a secretory product of the bacterium for a group of cells. To this last group of products is given the name of toxins.

It can be understood that a given bacterium can be pathogenic for one species of animal and not for another. All depends upon the degree of toxicity of the secretory products for the leucocytes of the species. It is the same for the special toxins. They are not toxic for a given animal species unless they possess an affinity, that is, a special coefficient of adsorption, for a corresponding group of cells.

It can likewise be understood that the reactions due to the colloidal disturbances brought about by the secretory products produced by bacteria of different species may manifest themselves variously, according to their different properties: for these products vary from one species to another. A chemical reaction always operates in an identical manner, but a colloidal equilibrium may be modified in an infinite number of ways without being inevitably destroyed, and, although modified, may continue in a different state.

It is evident on the other hand, that a species of bacteria, capable of being pathogenic for two different animal species, may provoke in each of them disturbances which are evidenced in different manners, for the products of secretion are unable to modify in exactly the same way two states of equilibrium which are not comparable. Furthermore, the metabolic products of the same bacterium will necessarily be different in accordance with the nature of the food, and this can not be identical if the bacterium develops in an individual of one species of animal or in a member of another species. There is much greater reason that these products will be different when the bacterium develops in an artificial medium. It is by no means astonishing that certain bacteria which secrete in the body substances possessing a violent toxic action do not secrete them in an artificial medium. The food in these two cases being markedly dissimilar as to its chemical nature, it is evident that the products of secretion can not be identical in the two cases.

The products resulting from the vital activity of a bacterium may therefore exercise a general action extending to all the fluids of the body, and a local action directed upon a certain particular group of cells. Every bacterial species pathogenic for a species of animal, exercises in general the two actions simultaneously, but with a very variable respective intensity. In so far as man is concerned, the general action is predominant in the case of the parasitic protozoa, the tubercle bacilli, the typhoid bacillus, for example, and the special action is preponderant in the case of the tetanus bacillus, while for the diphtheria bacillus and the toxic dysentery bacillus both actions are manifest.

THE REACTIONS OF THE ORGANISM

The reactions of defense on the part of the body differ to accord with what is produced by the bacterial products; whether they manifest their action by a disturbance in the general colloidal equilibrium, whether they exercise an action on the leucocytes, or whether a true toxin causes a specific disorganization involving a special group of cells.

No efficacious reaction of defense appears to be possible against the first—against products lacking special elective affinity. The

body is not able to respond to a simple loss in equilibrium of the colloidal state provoked by the production of antagonistic substances except by an adaptation to the new equilibrium. But this new physical state is of necessity very instable and subject to sudden rupture under the influence of a new invasion of the body by the same products. This explains why a serum has never been obtained possessing any curative or preventive power for a disease caused by a bacterium, which, in vitro, at least, secretes products which act physically only, having no elective affinity.

To fix these ideas, let us consider the two types of bacillary dysentery, the one caused by a toxic bacillus, the bacillus of Shiga, the other by the atoxic forms, the bacilli of Flexner and of Hiss. By the injection of a horse with cultures in the case of the toxic bacillus (or with toxin) a serum is secured which possesses a curative action, and this solely because it contains a specific antitoxin. On the contrary, the sera from horses prepared with the atoxic bacilli do not have any curative power whatever. In other words, the body of the horse has reacted to the presence of bacterial substances possessing specific toxic action by the production of an antitoxin, although no neutralizing substance is formed under the influence of the injection of bacterial substances without any affinity.

Against the bacterial products destructive for the white cells, that is to say, against those products which confer upon the bacterium its pathogenic property for a given species of animal, the susceptible organism appears to be able to react by the production of neutralizing substances, to which have been given the name opsonins. The properties of these opsonins are obscure, and they are not constant in the sera of immune animals. On the other hand, the leucocyte itself seems able, in certain cases, to acquire by adaptation, the property of engulfing and of digesting these pathogenic bacteria.

To the secretion by the bacterium of substances possessing a special affinity for a group of cells the body possesses a powerful means of defense. It responds by the production of antitoxins which neutralize the toxins. Here the reaction is absolutely clear.

Among all of these defensive reactions brought about by the organism against a bacterium, there are but two whose reality is proved, phagocytosis and the production of antitoxins. All of the other described reactions are either doubtful, or are manifested, and then weakly, only in particular cases.

Do there exist other endogenous means of defense as yet unknown? It is very possible. However that may be, phagocytosis alone explains perfectly all natural immunity which characterises the refractory state. As for the resistance acquired by virtue of the attack of a disease, the production of antitoxin, in its broad sense of substances capable of neutralizing toxic bacterial products, is sufficient to explain this immunity. Phagocytosis may then exercise itself on a bacterium henceforth inoffensive, because its harmful secretions have been neutralized.

Natural immunity and acquired immunity are, therefore, not of the same nature. In the first, phagocytosis takes place because the organism does not secrete substances toxic for the white cell. In the second, because the presence of the bacterium in the organism leads to the formation of principles neutralizing these toxic products, whether they are formed within the interior of the leucocyte, which in that case has acquired an adaptation to phagocytosis of the bacterial agent of the disease, or whether they are free in the fluids of the body.

But whatever may be the means of defense which the organism uses, we know definitely that *organic* immunity is not acquired rapidly; it requires a certain number of days, and sometimes even weeks, between the moment when the organism is invaded by the pathogenic bacterium and when immunity is established. This leads us to consider another phase of the question.

It is known that animals susceptible to a disease, not having acquired an immunity by virtue of a previous infection, do not always contract the disease when they are exposed to infection. Does there exist, then, a defensive process which protects a susceptible animal if it is invaded by a pathogenic bacterium? On the other hand, when an animal contracts a contagious disease, does it remain unarmed, at the mercy of the bacterium, up to the time when the immunity which emanates from its body itself, is developed? By no means. But here the defensive means is not derived

from the body itself. It is due to the presence in the bodies of all animals of an ultramicrobic parasite of the bacteria, the bacteriophage.

In summary, a bacterium having succeeded in penetrating into the body, two situations may develop. The substances secreted by the bacterium may or may not possess a toxic action for the leucocytes of defense. In the second case the bacterium is eliminated by phagocytosis, the animal is refractory. In the first case it is susceptible.

On the other hand, whether the animal be refractory or susceptible, it harbors normally in its intestine an ultramicrobe parasitic of the bacteria, capable of adapting itself by contact to bacteriophagy of the bacteria[1] which have succeeded in invading the organism. In the case of the refractory animal, the elimination of the bacterium by the ultramicrobe is not of great importance, for, if it is not exercised, phagocytosis will be adequate to assure the integrity of the organism. On the contrary, in the susceptible animal, this defense by the bacteriophage is fundamental and the issue of the struggle which takes place between the bacterium which defends itself and the ultramicrobe which is attacking, determines the fate of the individual in which the struggle takes place, each of the fluctuations of the struggle being reflected by changes in the condition of the patient. If the bacterium wins, the patient succumbs; if the ultramicrobe is victorious, cure results. It is only at this moment that the reactionary phenomena of the body itself enter in, in the case of the immunizing diseases, by the acquisition of an immunity which places the recovered animal for a longer or shorter time in a state comparable to that of the naturally refractory animal.

[1] Investigations up to the present time have been conducted only upon their action on the bacteria. Do they exercise a similar action upon the protozoa? It is not known. Experiments are very difficult to carry out as regards the protozoa, since, except for rare exceptions, they can not be cultivated in artificial media.

CHAPTER V

PHAGOCYTOSIS IN THE ANIMAL SERIES

While physicians were still holding to the theory of miasmata as an explanation of contagion the botanists and zoologists, in the middle of the last century, were determining the real causes of certain diseases found among the plants and lower animals. It is indeed interesting to note that the first contagious diseases scientifically studied were those affecting the protozoa. In 1855 Alexandre Braun proved that certain plants and flagellated infusoria, feeding after the fashion of vegetables by absorption of the substances dissolved in the medium, were invaded by minute fungi of the Chytridaceae family, which fixed themselves on the walls and absorbed the still living contents of their hosts. It has since been recognized that in this group of the flagellates, those which nourish themselves by endosmosis are very subject to infection by the Chytridiales, while, on the contrary, those which engulf and digest their living prey are, in general, free from infection.

In the amebae it is possible to perceive most readily the mechanism of the digestion of bacteria, which constitute together with the algae, their normal food. An ameba is formed of a small protoplasmic mass containing a nucleus. When a bacterium comes into proximity to an ameba, the latter extends a portion of its protoplasm in the form of an arm, a pseudopodium, which embraces the prey. Then the pseudopodium withdraws, retracts, reëntering the mass of the protoplasm, transporting with it the bacterium which is thus engulfed. Digestion occurs in two stages. A transparent vacuole, presenting a slightly acid reaction and containing the bacterium forms within the weakly alkaline protoplasm of the ameba. The bacterium is quickly killed, the reaction of the vacuole becomes alkaline, and true digestion begins. The nutritive substances contained in the bacterium diffuse into

the contents of the vacuole, and later the ameba opens outward rejecting the residue of the exhausted bacterial body.

Digestion in the ameba takes place through the production in the protoplasm of the ameba of a ferment, as shown by Mouton, capable of acting in acid, neutral, or alkaline media.

There is certainly an adaptation of the ameba to the digestion of its habitual prey, for if one feeds an ameba for a time with colon bacilli, they yield an extract capable of agglutinating colon bacilli and of dissolving them in vitro. This offers an explanation for the resistance to infection of the protista which are nourished by intraprotoplasmic digestion; they kill and digest the invaders. Their resistance may, however, be overcome by certain species, such as the Microsphaera, which are able to neutralize or inactivate the digestive action of the protoplasm of the ameba. The Microsphaera multiply in the interior of the ameba and bring about its death.

It has often been asked if immunity can be produced in the protozoa. A recent finding of Whitney answers this question in the affirmative. *Hydra viridis* is normally infested by a green alga, *Zoochlorella vulgaris*. But if, to the water in which they live, one per cent of glycerin is added the hydra expel the zoochlorellae, and from this time on they are not infected, even if brought into an aquarium containing infected hydras. The hydra once cured possesses an immunity.

The mode of intracellular nutrition of protozoa served as the point of departure for Metchnikoff upon which he built his theory of phagocytosis. In the course of a series of admirable investigations, following the animal series from the ameba to man, he showed that the endogenous defense against the parasite must be in all cases only a developmental result of the mode of nutrition of the ameba.

Among the least developed pluricellular beings, the Porifera and the lower gastropods, the processes of digestion are identical with those of the ameba. The cells lining the canals through which the water circulates in the first, the cells which cover the filaments of the gastro-vascular cavity in the second, engulf the solid particles present in the water and digest them. In these organisms nutrition is effected solely by phagocytosis.

Nutrition by phagocytosis is still to be found in the Planaria, the flat worms which suck blood. The ingested blood fills the digestive tract, the walls of which are covered by cells which act as do the amebae, throwing out pseudopodia to engulf the red blood cells. Digestion takes place within the protoplasm of the ameboid cell, which, when the ingested cell is exhausted, opens out again to expel the débris, which falls into the digestive canal and is rejected by the animal.

Higher up in the zoölogical scale this rudimentary mode of feeding is replaced by procedures much more complicated. The foods are digested in a cavity, the stomach, by the aid of ferments formed in specialized glands. The nutritive colloidal foodstuffs are broken down and pass into absorbable crystalloid states. But the phagocytes do not disappear; they are found in all types of animal in the blood and in several organs. Functioning as true amebae, they engulf such foreign particles as succeed in entering the body.

THE PHAGOCYTES

The white cells, that is the leucocytes, of the blood of verte-brates represent the phagocytes of Metchnikoff. These are color-less cells, of which there are two principal types, the mononuclear leucocytes and the polynuclear myelocytes.

The mononuclear leucocytes are of two classes, the lymphocytes or small mononuclears, and the large mononuclears. The lympho-cytes are small elements, 6 to 7μ in diameter, and they represent in man 23 per cent of the total leucocytes. They originate in the lymphatic glands. They have a large nucleus, surrounded by a thin layer of protoplasm, and their rôle in phagocytosis appears to be negligible.

The large mononuclears, with a diameter of 8 to 9μ, have a vesiculated nucleus, with an irregular contour, and they possess an abundant protoplasm without granulations. They are pro-duced by the lymph glands and by the spleen and represent nor-mally 2 to 6 per cent of the total leucocytes. They are the mac-rophages of Metchnikoff.

The myelocytes include three classes of elements, termed the basophils (0.5 per cent), eosinophils (2 to 4 per cent, and the neu-

trophils (65 to 70 per cent), according as their granulations are stained by basic or acid dyes. The myelocytes form within the bone marrow. They are often termed polynuclears, although they possess but a single nucleus which presents lobes strongly divided giving the impression of multiple nuclei. The polynuclear leucocytes measure 9 to 10μ in diameter and have an abundant protoplasm. They are the microphages of Metchnikoff. The doctrine of phagocytosis, as established by Metchnikoff, is, however, too exclusive. From the work of different authors, chiefly those of the American school (Mallory, Lewis and McCoy, Buxton and Torrey, Gay and Morrison, among others) it appears that all of the ameboid cells of the body intervene in the process of phagocytosis. Particularly involved are certain connective tissue cells, the clamatocytes of Ranvier, and the endothelial cells of the blood capillaries and the lymphatics. These endothelial cells play a fundamental rôle in all of the phenomena of immunity.

THE RÔLE OF THE MACROPHAGES

In animals which undergo metamorphoses Metchnikoff has shown that phagocytosis plays an important rôle in the resorption of the tissues which disappear. This is a very general fact; it is by phagocytosis that the tail is eliminated from the tadpole, and that the larval tissues disappear in the Insecta; it brings about the resorption of cartilage at the beginning of ossification, the regression of the cells of the thymus in infancy, and the involution of the post-partum uterus in the higher vertebrates. In old age, the senile evolution of the ovary and the regression of the nervous elements are the work of the phagocytes.

As long ago as 1870 Langhans observed that the resorption of cellular elements of sanguinous effusions was effected by phagocytic action; the cells were engulfed and digested. It is the same for the cells of damaged tissues.

And finally, the macrophages are, as we will see, the cells which engulf and destroy the microphages which succumb in the struggle against the bacteria.

INFLAMMATION

We owe to Metchnikoff a whole series of investigations concerning the origin of inflammation, a process which results from

traumatism or from the penetration into the tissues of any object, animate or inanimate.

He introduced a splinter of wood into the gelatinous tissue of a medusa and saw, some hours later, the accumulation about the foreign body of a great number of ameboid cells, which rendered opaque the normally translucent tissue. Why this collection of cells? If the splinter was powdered, prior to its introduction, with a fine colored powder such as carmine or carbon, it was seen that the grains of the powder were engulfed and transported in the tissues, clearly proving that the calling together of the ameboid cells has as an object the engulfment and elimination of foreign particles.

If we go higher in the animal series we encounter always this same process. In the vertebrates, and in general in all animals having a circulatory system, there occurs at first a vasodilation which appears to be under the regulation of the nerve cells distributed in the walls of the vessels, the flow of blood is retarded, and there occurs, along with an exudation of plasma, a passage, by diapedesis, of leucocytes through the vascular walls. Such is the prelude to phagocytosis. The leucocyte is then found in the vicinity of the element to be eliminated. Thanks to the possibility of diapedesis the process of phagocytosis, which normally takes place within the blood, can be effected within any tissue or in the body cavities.

Inflammation, which formerly was considered a disease, is then a reaction of defense, leading to the elimination by phagocytosis of the irritating body implanted in the tissues, whether this irritating substance be an inert splinter or a living body. But in the latter case the phenomenon may be more complicated, for the living being resists the digestive secretions of the phagocyte; indeed, the rôles may even be reversed, when the parasite is able to secrete ferments which permit it to utilize for its development the protoplasm of the leucocyte itself.

CHEMOTAXIS

The first act of phagocytosis consists in an attraction which places the leucocyte in contact with the foreign particle. This phenomenon has been termed chemotaxis, and it has been subjected

to experimental study, for the leucocytes continue to live for some time when outside of the body. To procure them it is sufficient to inject some bouillon into the peritoneal cavity of a guinea-pig, and the exudate, upon removal a few hours later, will be found to contain a very great number of leucocytes.

The leucocyte is capable of motion. Placed upon a slide, microscopic examination shows that the protoplasm throws out prolongations which spread out on the glass, then, seeming to take a point of fixation in the pseudopodium, the remainder of the protoplasm slides, or rather flows, in its direction. The result is a change of position. Anesthetics abolish this tactile sense.

The direction of the motion may be influenced by the conditions of the medium. This is, moreover, an essential property of living matter which is manifested in the most diverse beings. Pfeiffer has shown, for example, that the spermatozoa of ferns are attracted by the malic acid secreted by the female organs, and that this attraction increases with the concentration. In the same way green algae placed in a liquid lighted by rays broken up by a prism, direct themselves toward the end of the spectrum containing the luminous radiations best adapted to their use.

Against the bacterial secretions the leucocytes react in very different manners; attracted by some, they are repelled by others. The reaction to the tetanus bacillus offers a remarkable example. Vaillard introduced under the skin of a guinea-pig some washed tetanus spores, that is, spores free of toxin. The phagocytes collected, engulfed the spores, and the disease did not occur. These spores are not always digested, but may be transported into certain organs, the spleen in particular, where they persist for a very long time. Vincent has shown that it is only necessary to inject a salt of quinine, a substance which possesses a toxic action upon the leucocytes, to cause the spores to germinate, bringing about an attack of tetanus. Instead of injecting washed spores, the guinea-pig may be injected with spores impregnated with toxin; the positive chemotaxis of the former case becomes negative, the spores are not engulfed, they germinate, and disease results.

With regard to the anthrax bacillus, Metchnikoff has shown that if a culture attenuated by the method of Pasteur is inoculated

into the ear of a rabbit there is a mobilization of the leucocytes at the point of injection and inflammation results. The bacteria are phagocytized and the animal is not affected. On the contrary, if a virulent culture is introduced, a vasodilation occurs it is true, together with an infiltration of plasma, but diapedesis does not take place. The bacteria multiply and the animal succumbs.

The same phenomena are reproduced in hemorrhagic septicemia of cattle. The injection of a virulent culture is followed by the formation of an enormous edema entirely lacking in leucocytes, and the animal dies. With an attenuated strain there is a small edematous infiltration, rich in leucocytes, and the animal lives.

ENGULFMENT

With the exception of the bacteria which secrete products which cause a repulsion, all foreign bodies which may enter the tissues undergo phagocytosis, whether they are motile or non-motile. The phenomenon can be followed in vitro, since, as we have said, the leucocytes remain alive for at least twenty-four hours after their removal from the body. The engulfment of a particle, animate or inanimate, by the leucocyte follows a method identical with that which we have seen taking place in the case of the ameba. The most curious spectacle is provided by the phagocytosis of a trypanosome.[1] By means of pseudopodia the leucocyte seizes upon the protozoan at one of its extremities, and incorporates it slowly into its protoplasm, while that portion of the animalcule which is still free moves violently, manifesting thus its repugnance to engulfment.

Substances in solution, such as alkaloids and the toxins, can also be fixed by the leucocytes. Here, it is not an engulfment but a special affinity of the dissolved body for some of the substances of which the leucocytic body is composed. This action can, in certain cases, afford protection for the body. The leucocyte,

[1] Blood flagellates, very motile, flame-shaped, elongated, and provided with an undulating membrane. They are 20 to 30μ in length, that is, 3 to 5 times the diameter of a leucocyte. These are the agents of different animal diseases and of human trypanosomiasis, or sleeping sickness.

which is replaced if it succumbs, diverts the toxin and prevents its union with a fixed cell.

DIGESTION WITHIN THE LEUCOCYTE

It appears, therefore, to be fundamental, that all bacteria which exert a negative chemotaxis toward the leucocytes of a given animal are pathogenic for this animal. Free in the blood or in the tissues, these bacteria develop. But the reverse of this is not true; a bacterium causing a positive chemotaxis may or may not be pathogenic, depending upon the manner in which the bacterium engulfed by the leucocyte acts. The engulfment is often only the first skirmish of the struggle.

Let us examine first what goes on in the case of the engulfment of an inoffensive bacterium. Microscopic examination shows sometimes that the bacterium first undergoes a deformation, it becomes swollen, globular in form, resembling that which takes place in the case of the vibrios. Cocci often dilate and increase in volume. In other cases the bacterium maintains its form, but fragments. Complete digestion is a relatively slow process, requiring some hours or even several days.

If one tests the reaction of the phagocytized bacterial body by means of dyes one sees that the normal alkaline reaction becomes gradually acid. This acid reaction is transitory, and gives place after a few hours to a weak alkalinity. This is a general fact; the processes of digestion appear to be the same as in the ameba.

It is but natural that attempts have been made to isolate the digestive principles of the leucocyte. The results thus far secured have not been entirely satisfactory, but from these investigations it appears that the true ferments of the leucocyte are extremely fragile, and are only able to manifest their action when within the interior of the phagocyte.

RESISTANCE TO PHAGOCYTOSIS

We have seen that a bacterium of an attenuated strain is phagocytized and that this does not take place with a virulent strain. Why this difference in attitude on the part of the leucocyte? Experiment shows the following.

Wollmann, working with an attenuated anthrax bacillus culture, proposed to separate, to select, the germs readily phagocytized from those which were resistant. This was accomplished by injecting such a culture into the peritoneal cavity of the guinea-pig. After a few hours the peritoneal exudate was removed and the mixture was centrifugated. The leucocytes with the bacteria which they had engulfed collected in the bottom of the tube, the free bacteria remained in the supernatant fluid. Inoculations made with bacilli which had been phagocytized but not killed gave a growth, as did the inoculation made with the free bacilli. Guinea-pigs were injected with each of these two cultures, and the procedure of separation was repeated several times. After a number of passages two strains were finally obtained with very distinct properties; one strain was readily engulfed by the leucocytes, the other was completely refractory, and the latter was further distinctive in that the bacteria were surrounded by a mucoid capsule. The fact that capsules exert a protective action had been known for a long time. Bordet had observed it with streptococci in 1896, but in the experiment of Wollmann the influence of the capsule is particularly sharp.

Here again we find a mucin, a substance analogous to that which protects the cells of the lining of the digestive tract against the action of the ferments. Biologic processes are not very varied after all. We have seen that phagocytosis is operative from the protozoa up to man; we see now that the system of protection for the bacterial cell or for the human cell against the action of dissolving ferments involves the secretion of comparable substances. We will see the same defensive procedure again in the bacterium, in opposition to its parasite, the bacteriophage.

This protective procedure is not, however, the only one which the bacterium can utilize. Not all engulfed bacteria become the fatal prey of the leucocyte. Many bacteria, although secreting principles which induce an energetic positive chemotaxis on the part of the leucocyte, since they are habitually found enclosed in their protoplasm, are not, however, digested. They produce substances capable of neutralizing the action of the leucocytic ferments. Certain ones are even able to secrete digestive ferments, thanks to which they are able to utilize for their development the leucocytic

substance. Such are, among others, the gonococcus, the meningococcus, the staphylococcus, and the streptococcus.

It appears, then, that a bacterium is able to resist phagocytosis in different ways; sometimes they secrete products which repel the leucocytes, sometimes they surround themselves by a protective capsule, and sometimes they produce ferments which permit them to utilize the substance of the leucocyte as a food.

THE RÔLE OF PHAGOCYTOSIS

Even before the studies of Metchnikoff it was known that the leucocytes are able to engulf bacteria, but it was not known that they are capable of digesting them. They were considered solely as conveyors. For Metchnikoff, phagocytosis became, in all cases, the sole protection of the body against bacterial invasion. The truth is to be found between these two extremes. A bacterium is pathogenic for a person if it resists phagocytosis, either because it causes a negative chemotaxis on the part of the leucocyte, or because, although engulfed, it resists digestion, and it is precisely the possibility or impossibility of phagocytosis of a given bacterium which determines whether or not the organism is pathogenic. But phagocytosis is powerless against a number of bacteria, which, because of this fact, are able to grow in the body. In certain cases the leucocyte is able even to play a harmful rôle, as when it introduces organisms which resist phagocytosis into the body— the tubercle bacillus and the meningococcus, among others. Here, it is a carrier.

The leucocyte is not the providential agent of Metchnikoff. If the bacterium has toward the leucocyte a positive chemotaxis, resulting from a physical phenomenon, the bacterium is phagocytized, quite without reference as to whether or not the leucocyte is able to digest it. If it digests it, the action quite truly tends toward the maintenance of life, if it does not digest it the action tends toward the termination of life.

In many infectious diseases there can exist no question but that the bacterium, an indigestible parasite, exerts a positive chemotaxis upon the leucocyte, for example, as in the case of tuberculosis, and cerebrospinal meningitis.

Meningitis begins by a benign rhino-pharyngitis, but the mucous membranes, especially at the level of the tonsillar ring are traversed by an abundance of leucocytes which creep over the surface. These leucocytes engulf the meningococci, pass through the mucous membranes with their prey, and enter the lymphatic system and then the blood. They introduce the parasite into the body, and since it is the parasite which finally digests the leucocyte the pathogenic bacterium is liberated within the body.

In tuberculosis, in the great majority of cases, penetration into the body is accomplished in the same way through the aid of a leucocyte passing through a mucous surface.

Nothing in nature is effected in view of a determined end. In the body reactions are produced, in the physico-chemical sense of the word. Certain of these reactions tend to maintain the instable equilibrium of the colloidal complex which in reality is the body, others tend toward its rupture.

Phagocytosis intervenes as a process tending to maintain life only in those cases where the leucocyte is able to digest the bacterium, and it is precisely because of this that the refractory state which characterizes the *natural immunity* of an animal species depends upon phagocytosis. An animal species is naturally refractory to a disease of which the causative agent is always phagocyted and digested by the leucocytes of animals of this species. Inversely a bacterium is pathogenic for an animal species if it exercises on its leucocytes a negative chemotaxis, or if, when phagocyted, it resists leucocytic digestion. Such a bacterium grows within the body and disease breaks forth.

In the immunity following recovery from an infectious disease phagocytosis is likewise operative. It may be activated because of a property acquired through adaptation of the leucocyte, which, by practise is habituated to secrete ferments which permit the digestion of the formerly resistant bacterium; or, more frequently, it is because of the formation in the body of one of those substances, which, by neutralizing the secretions of the bacterium, render it more vulnerable.

The adaptation of the phagocyte does not appear to play an important rôle in the establishment of acquired immunity, as seems to be shown by the following findings. The diseases

caused by those bacteria, which, although exerting a positive chemotaxis, multiply within the leucocyte itself, are precisely those diseases which are liable to recur at short intervals. Such are the infections caused by the gonococcus, the staphylococcus, and the streptococcus. On the other hand, Besredka has noted a curious fact. If one injects into the peritoneal cavity of a rabbit a culture of anthrax bacilli, taking care not to contaminate the skin of the animal, the rabbit does not contract the disease, nor does it develop an immunity, despite the fact that the bacteria have been phagocytized. The simple fact of phagocytosis is not sufficient to induce adaptation.

However that may be, this adaptation certainly must occur, even though it persists for a limited time only, and to this is due that feeble and transitory immunity resulting from an infection caused by the staphylococcus and analogous organisms.

If phagocytosis takes place in the course of *acquired* immunity, it is because there exists in the humors of the organism a substance which paralyzes the defense of the bacterium. It is known that the blood of immunized individuals shows the presence of opsonins which may play such a rôle. But what is an opsonin? We will see that the bacteriophage, of which the defensive rôle is preponderant in the susceptible animal secretes powerful opsonins which necessarily intervene in the course of the processes of recovery, but the presence of these opsonins does not explain the acquired immunity, for they disappear during convalescence. It seems that, in the last analysis, in acquired immunity an opsonic action may be exercised by the antitoxins.

EXPERIMENTALLY ACQUIRED IMMUNITY

The immunization of susceptible animals may be obtained, as we will see, by the method of vaccination with attenuated viruses, as discovered by Pasteur. The immunity thus acquired is solid and durable; it is essentially equivalent to that which results from a benign attack of the natural disease, since the attenuated virus, in effect, produces the attenuated disease.

We have seen, likewise, that it is possible to immunize thoroughly by injections of antitoxic sera. This immunity is passive and of short duration—twenty days at most.

Sometimes a mixed method is used, which is effective in the vaccination of animals against ultramicrobial diseases, such as sheep-pox, and bovine and porcine plagues. One injection of serum permits the animal to support an inoculation of the virus.

There is finally, the method of vaccination by means of killed bacterial bodies, of which the type is antityphoid vaccination. What is the real value of this method?

It is certain that in some cases the injection of bacterial bodies can produce an immunity, probably based upon an adaptation of the phagocytes. This immunity is of greater or less duration, but probably does not last longer than six months in the most favorable case, that of the cholera vibrio. And it is none the less sure that the method is not of general application, as is evidenced by the complete failures recorded for a number of diseases, for the prophylaxis of which vaccination by killed bacteria has been recommended. Such is the case, notably, for the pneumococcal infections and bubonic plague, but the most interesting case is that of vaccination against typhoid fever.

It is significant to recall that Metchnikoff, after having shown that the chimpanzee is susceptible to typhoid fever, tried to test with this susceptible animal the efficacy of antityphoid vaccination. The results of his studies were that vaccination by living bacilli was alone efficacious; the repeated injection of typhoid cultures killed by heat or by ether did not lead to any evidences of immunity, the chimpanzees remaining as susceptible as before.

To this experiment, of which the result is in the negative, statistics reply by an affirmation, and the scientific world has quite generally concluded in favor of the method. What is the truth?

The facts observed with regard to man furnish material for discussion. During the course of the recent war, for example, vaccination has not been the sole measure of prophylaxis employed in the campaign against typhoid, and it is difficult to allocate the respective rôles in the prevention of the disease of, on the one hand, the vaccination, and on the other, the prophylactic measures, such as the use of wine as a beverage, and the control of water used in foods, directed toward preventing the ingestion of the pathogenic bacteria. It is possible, however, by an indirect method, to evaluate their respective efficiencies.

Before the era of prophylaxis the great disease of armies, of an importance much greater than typhoid fever, was bacillary dysentery. Infection in the two diseases is brought about in an identical manner, and all prophylactic measures, vaccination excepted, applied during the last war were as efficacious against infection with the organisms of the one of these diseases as against the other. But, in the different armies involved, the morbidity from dysentery was extremely small, even lower than the *official* morbidity given for typhoid fever. What could not have been said for the efficacy of an antidysentery vaccine had it been used? But the armies were not vaccinated against dysentery. If epidemics of this disease did not decimate the armies, as has always been the case in preceding wars, it is because the prophylactic measures directed toward the prevention of contamination were effective, and as these measures are the same for typhoid fever and for dysentery, there can be no doubt that it is to them, in great part, that we owe the relative low morbidity from typhoid.

What shall we conclude? The question remains open.

When all of the barriers are broken down, the pathogenic bacterium unsusceptible to phagocytosis being present in the body, disease manifests itself.

Each bacterial species produces a specific action which expresses itself in the symptoms and in the lesions which differ for each species. We have already touched upon this subject in the chapter dealing with infection; let us return to it briefly.

In the first place, there certainly occur chemotactic phenomena between a bacterium and the different tissues. In no other manner can the localizations, often very specific and constant for a given bacterial species, be explained. Such is the case, among others, for the meningococcus which always grows in the cerebro-spinal fluid; for *B. diphtheriae* which localizes upon the pharyngeal mucosa; for *B. dysenteriae*, localizing in the intestinal tract. Other bacteria are less elective. *B. tuberculosis* may develop in any tissue whatsoever. This question of localization plays naturally a preponderant rôle upon the nature of the lesions and upon the local symptoms of the disease. But there is another cause which is very important, which is preponderant in so far as the general symptoms and the acquisition of endogenous immunity are concerned.

The diphtheria bacillus localizes upon the mucosa of the upper respiratory tract, and this localization causes the angina, but the fundamental action of the organism is upon the nervous centres. The tetanus bacillus remains localized at the point of inoculation, where very often no lesion is produced, all of its activity being likewise manifested upon the nervous centres.

As regards immunity, certain diseases provoke the establishment of a refractory state of long duration, with others the immunity is slight and transitory. Note particularly that the immunity, at least that immunity which is strong and durable,

only occurs in those cases where the cells are directly influenced, either by the parasite growing within the cells themselves, as is the case for the ultraviruses against which the immunity is of the greatest degree, or where the bacterium elaborates a product, a toxin, which acts upon a group of cells. I simply note this distinction in passing; we will return to it.

In disease there is a multiplication in the body of the bacterial substance, a protein substance foreign to the body. We have already seen the nature of the disturbances in equilibrium which may take place in the colloidal complex of the body in protein shock and in anaphylaxis. But this foreign substance is living, it assimilates. The bacterium withdraws from the medium, that is, from the tissues, the elements which provide it with the energy necessary to life and for the building up of substance of its own kind. But more than this, and very important it is, it discharges into the medium the products of its metabolism.

The bacterial substance is different for each species of microorganism, and the products of metabolism are likewise different, even different for a single species in accord with the nature of the food supply. These substances and these products fix themselves upon different cells, according to the coefficient of adsorption of each species of cell for each of the products, these localizations determining the diversity of symptoms and lesions and the diversity of the reactions of the cells since the reaction is always specific and is adapted to the rhythm of the excitation.

We have seen in what the adsorption consists. Each cell is an adsorbant; each of the products distributed into the medium by the bacterium is adsorbed, but in variable proportions by the different types of cell. The coefficient of adsorption of each species of cell is different for a single bacterial product. The coefficient of a given type of cell is different for the different products secreted by a given bacterium, and with greater reason for the products secreted by bacteria of different species.

That which we term toxin is a bacterial product for which a class of cells possesses a special coefficient of adsorption. Against tetanus toxin the cells of the nervous system react like silk to malachite green. They fix almost completely the toxin liberated by the bacillus and conveyed to them by the blood, while other

cells, having but a weak, or no coefficient of adsorption fix it only slightly or not at all.

But each cell is a complex colloidal system. All foreign substances adsorbed lead to a disturbance in equilibrium of this system, and if the modification in equilibrium rapidly reaches the point of irreversibility, that is to say, without there being time for adaptation to take place, disorganization results, with the death of the cell, representing a partial death of the whole organism. If the number of partial deaths is sufficiently great to lead to the cessation of an essential function complete death follows.

When the number of cells possessing a high coefficient of adsorption for a bacterial product is small, and when the coefficient of adsorption by these cells is very great, the probability of death of the invaded organism will be correspondingly great. In man, the mass of nervous cells possessing a coefficient of adsorption for tetanus toxin is but a few grams, or about a ten-thousandth part of the weight of the body. Practically all of the toxin secreted by the bacteria concentrates in these few grams of cells and causes inevitably a rupture of equilibrium which rapidly reaches the irreversible point. In this instance, since these nervous cells play an essential rôle, their death leads to complete death.

In tuberculosis, no class of cells appears to possess a special coefficient of adsorption for the bacillary products. Each of the cells of the body fixes only a minimal quantity of these products. Moreover, since the general coefficient of adsorption is weak the major part is eliminated by the natural emunctories. Under such conditions, micellar adaptation is able to take place. Throughout the organism a new equilibrium is established, compatible with the general coördination of cellular functions. But the functions of each cell, solely determined by their actual state of equilibrium, are not identical to those which were present prior to the disease. The tubercular individual is a being in process of symbiotic adaptation. When death takes place it is not however, the result of an irreversible modification directly caused by the action of bacterial products, but by an exaggeration of the reactional processes of its own cells, the leucocytes in particular, of which the principle manifestation is the tubercle, and death takes place

primarily because of a functional insufficiency of the lungs or of the organs attacked. In tuberculosis, the less violent the reaction the longer the survival. It is by no means an impossibility that, in a distant future, the symbiosis will become perfect. With regard to this bacterium man may become a symbiotic being, differing in certain respects from the present-day man, adding one more to the many examples of symbiosis found in the animal and vegetable kingdoms.

Every bacterial species secretes not one, but several products and each of these products is adsorbed in variable proportion by the different cellular groups of the individual. The general symptoms of a disease and the cellular reactions which take place are regulated by the respective coefficients of adsorption of the different cellular gels for the bacterial products.

HUMORAL THEORIES

The humoral theories were the first, in point of time, to be advanced to explain the resistance to contagion possessed by persons naturally refractory and by those who, although susceptible, became refractory as a result of a first attack. They explained the refractory state of the organism, either by virtue of the presence in the humors of certain dissolved substances possessing bactericidal properties, or by the fact that the physico-chemical properties of the plasma were opposed to the development of the bacterium.

Pasteur advanced the hypothesis that the bacterium is pathogenic, that is to say, that it is able to develop in the organism, when it finds there certain appropriate nutritive principles. These principles withdrawn, the bacterium is unable to develop a second time in the same organism. This is the theory of exhaustion.

For Chauveau, there exist in the organism, naturally immune or having an acquired immunity, inhibiting substances secreted by the bacterium itself. This is the theory of addition.

Bouchard, Charrin, and Roger thought that the bacteria were able, without being killed, to be attenuated by the humors of the body to the point of becoming inoffensive. This is the theory of attenuation.

Previous investigations have shown that these hypotheses are in contradiction to the facts; they have therefore been abandoned and are of historical interest only.

The discovery of alexin by Buchner allowed the formation of a theory, still in high favor, explaining immunity through the agency of the bactericidal properties of the blood. The later discovery of agglutinins and of amboceptors seemed to confirm this. Nevertheless, all experimental fact and all observation shows that such a theory is without foundation. It is needless to return to the experiments which have been mentioned in the chapter treating of the reaction against colloids, and which show that this theory is inadmissible.

The blood of refractory animals which enjoy an immunity, either acquired naturally or conferred by vaccination, is, in general, incapable of killing bacteria. In the rare instances where the blood does possess such an action for a given bacterium, the animal may possibly be extremely susceptible. For example, while the serum of the dog does not possess any bactericidal property for the anthrax bacillus, and the serum of the rabbit is strongly active, nevertheless this last animal is very susceptible to anthrax, while the dog is refractory.

The essential condition for the acceptance of a theory must be that it accords with the facts. All theories which attempt to explain immunity through the agency of bactericidal substances present in the blood are contradicted by experiment and by observation, and are of necessity false.

ANTITOXIC IMMUNITY

The toxins

To Roux and Yersin we owe the discovery of diphtheria toxin. Filtering through a bougie a bouillon culture of diphtheria bacilli they obtained a liquid of which a very small quantity injected under the skin of the guinea-pig reproduced a disease identical with that provoked by the injection of living bacilli. They reached the conclusion that the harmful action of the bacterium was due to its secretions. It has been possible, by perfection in technic, to obtain toxins active in 0.004 cc., that is to say, that this quantity kills a guinea pig of 300 grams in four days.

Knud Faber, by a method identical with that of Roux and Yersin, next revealed tetanus toxin. Tetanus toxin possesses a very singular property. Endowed with a very great affinity for the nerve cells, it reaches the nervous centers by advancing along the fibers. Marie injected a lethal dose of toxin in the middle part of the tail of a certain number of rats, then sectioned the tails at the base after variable intervals of time. All of the rats whose tails were cut off in less than forty minutes after the injection survived, all of those which were not operated upon until after a longer time succumbed. He proved, by insertion of the nerves of the amputated tail under the skin of different mice, that in the first the toxin was still present, for the mice died of tetanus, while in the second, there was no longer any toxin, for it had passed toward the nervous center along the nerves.

Bacillus botulinus, the agent of intestinal food poisoning, likewise secretes a powerful toxin (Van Ermenghem), and this is also true for a number of other bacteria.

Bacteria are not the only organisms capable of secreting toxins. Everyone knows of the vegetable toxins, such as ricin, which is found in the castor-oil bean. The venoms of snakes contain complex substances among which are true toxins; cobra venin owes its properties almost exclusively to a neurotoxin.

What is the intimate nature of these toxins? It is not yet known; all that it is possible to say is that they are colloids. They become attenuated when they are exposed to air; light affects them in the same way. They are very sensitive to the action of heat, acids, hypochlorites, and iodine. Their properties resemble, then, those which characterize the ferments. As is the case for the latter, it has been impossible up to the present to obtain them in a pure state; they accompany the precipitates which form in fluids which contain them.

Very many pathogenic bacteria do not seem to be able to produce toxins in vitro, or, at least, the quantity formed is inappreciable. With certain of them it is possible to obtain a toxin by maceration of the bacterial bodies, and such principles have been designated endotoxins, in opposition to the exotoxins, which, like the toxins of diphtheria and tetanus, diffuse into the fluid. This difference of terminology as Nicolle has remarked,

does not correspond with a real difference in character. A more or less great diffusibility can not suffice to establish such categories.

We have said above that many pathogenic bacteria do not produce in vitro appreciable quantities of toxin; is it the same in vivo? Hardly probable, for with the vibrio of cholera[1] for example, the reactions seen in the disease are only to be explained as a result of a powerful toxic action, but, in vitro, no evidence of a cholera toxin can be found. This fact is explained if it is considered as a product of metabolism. The condition is analogous to that of the digestive bacterial ferments which differ markedly in accordance with the nature of the food utilized. This is so true, that we may see experimentally, a toxogenic bacillus furnish a good toxin in one medium and not give any at all in a medium of another composition. A bacterium may secrete a powerful toxin in the body and produce nothing in the culture media of the test tube.

The antitoxins

Maurice Reynaud was the first who, in 1877, had the idea of testing whether the blood of immunized animals possessed a preventive property. He took the blood of a heifer presenting the pustules of vaccinia which had developed for six days and injected it into a normal heifer. Fourteen days later this animal, vaccinated by scarification, did not take the vaccinia.

In 1890 Behring and Kitasato, preparing laboratory animals by a series of injections of diphtheria toxin, showed that the blood of these animals had developed an antitoxin capable of neutralizing the toxin. At the Congress of Budapest in 1894, Roux made known the results which he had obtained in the treatment of human diphtheria. The discovery of Roux was not, however, simply an application of that of von Behring. The properties of the antitoxic sera depended both on the quality of the toxin which was injected and the animal into which it was injected. Von Behring had injected relatively inactive toxins into guinea-pigs, and the serum so obtained was too weak to give marked results in the treatment of human diphtheria. Roux, who had

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[1] If indeed, the vibrio is the agent of cholera, a fact which has never been demonstrated.

worked with the toxins for several years, with L. Martin, perfected a technic of preparation, and this permitted him to obtain a very active toxin, and on the other hand he selected as the producer of antitoxin the horse, the animal of choice because it reacts strongly and furnishes potent antitoxins. In a word, this great discovery was made at three times; Roux and Yersin discovered the toxins, von Behring and Kitasato the antitoxins, and Roux and Martin the technic for the preparation of therapeutic antisera.

The horse, like all other vertebrates, is very sensitive to diphtheria toxin. The injection of a fraction of a cubic centimeter may cause death. It is necessary then to accustom the animal to support progressively larger and larger doses. In practise the immunization of the horse is effected by first injecting a very small amount of toxin attenuated by contact with iodine water. The injections are repeated every two days, gradually augmenting the quantity of the mixture and simultaneously reducing the proportion of iodine water. After about 20 injections the horse supports a small dose, a fraction of a cubic centimeter of pure toxin, and from this time the doses are rather rapidly increased. The thirtieth injection may be 30 cc., the fortieth, 200 cc. One thus obtains in three months sera titrating to from 100 to 300 units.

A more rapid method has been devised by W. Park. The first two injections are made with a mixture of toxin and antitoxin, followed by a series with the pure toxin. At the fifteenth injection it is already possible to inject 500 cc. In this way potent sera can be secured, of which the titre, according to the horse, varies between 100 and 1000 antitoxic units.

Recently Ramon has shown that if one adds to a toxin, diphtheria toxin for example, a minimal quantity of formol (0.3 to 0.4 per cent of commercial formalin) after a few hours at 40° to 42°C. the toxin loses all of its toxic property. A diphtheria toxin originally killing a guinea-pig in a dose of 0.00125 cc. is then tolerated in the enormous dose of 6 cc. But, Ramon has shown that this "detoxicated" toxin retains its immunizing power. A guinea-pig after two injections of 1 cc. withstands with impunity several thousand lethal doses of fresh toxin. A horse which received two injections of 1 cc., then 3 cc. of detoxicated

toxin yielded a serum possessing 6 units of antitoxin, and by repeating the injections with increasing amounts readily reached a value of 250 units. These experiments show that toxicity is independent of antigenic power.

One might predict that active immunization of man and of animals against toxic diseases might be easily accomplished by means of detoxicated toxin. The procedure would certainly be superior to that which consists in inoculating mixtures of antitoxin-toxin with a slight excess of the latter.

At each injection of toxin the body of the horse reacts with a production of antitoxin, in proportion to the quantity of toxin injected.

The animal is bled on about the eighth day after the last injection. In certain countries the horse is completely exsanguinated, giving thus about 12 liters of serum. At the Pasteur Institute only about 6 liters of blood are removed, furnishing 3 liters of serum after the retraction of the clot, then four days later 6 liters are again taken. Thirteen days later the horse receives an injection of 300 cc. of toxin, and four days later 500 cc. and bleeding is re-commenced after an interval of eight days. Certain horses are in this way able to produce a good serum for several months, exceptionally through several years. In other animals the antitoxic titre falls rapidly, despite injections of the toxin. It all depends upon the individual, which is very variable. Fairly often animals are encountered which react poorly to the injections and the antitoxin is formed in too small amounts to give a serum suitable for use. Horses of fine races are those which in general react the best.

The other antitoxic sera, antitetanus, antidysentery, antimeningococcic are obtained by similar procedures.

Properties of the antitoxins

The antitoxins are relatively stable at room temperature, but more so at ice-box temperature. The antitoxic titre of horse serum diminishes slowly, and several years are required for it to be reduced to one-half of its initial value. On the contrary, an increase of the temperature to 60° destroys it quickly and almost completely.

In serum deprived of water by concentration at a low temperature antitoxin can be preserved indefinitely without change.

Up to the present time antitoxin has not been isolated; it *seems* to be related to the fraction of the serum proteins which are termed the globulins.

Buchner has advanced the hypothesis that the antitoxin is a product of the transformation of the toxin. For von Behring and Ehrlich it is formed anew by the cells under the stimulus provided by the toxin. They base this hypothesis principally upon the disproportion between the quantity of toxin injected and the amount of antitoxin produced. All authors have gradually rallied to this last hypothesis. The last word has not, however, been said on this question, for the arguments adduced are not entirely free from criticism.

The mode of action of the antitoxins on the toxins has served as a subject for lengthy discussion. Ehrlich has wished to see a neutralization of the chemical order, Arrhenius and Madsen a chemical combination, instable and reversible, obeying the law of masses which govern the combinations effected between a weak acid and a strong base. For Bordet, it is simply a physico-chemical action.

Undoubtedly this last conception is the true one, for the first two, the first especially, are contradicted by experimental facts. It is needless however, to consider the many developments bearing on these discussions, which moreover have only an historical interest. M. Nicolle has given the direct proof that a flocculation takes place when antitoxin reacts with toxin. The recent experiments of Ramon confirm and more accurately fix those of Nicolle. Ramon has even devised a method for titrating antitoxin based upon the flocculation of a definite quantity of toxin, a procedure actually employed in many laboratories which prepare diphtheria antitoxin, for it is as sensitive, more expeditious, and more economical than the older method of titration (Ehrlich) by injecting guinea-pigs with mixtures of toxin and antitoxin. It may then be considered as settled that the toxin-antitoxin union is a colloidal reaction, the neutralization resulting from the formation of a flocculable complex.

Here again we see that the biologic reactions are not of the molecular order. All theory, purely chemical, is necessarily false when it is applied to phenomena which pass beyond the borders of molecular chemistry. The smallest possible particle of a "biologic" compound is not the molecule, but the micella.

Antitoxic sera

Up to the present time sera have been obtained for neutralizing the toxins produced by the diphtheria bacillus, *B. tetani*, *B. botulinus*, *B. dysenteriae*, *B. perfringens*, *B. oedematiens*, and the meningococcus. With reference to animal diseases sera have been obtained possessing preventive and curative properties for swine erysipelas and for certain animal diseases caused by the ultramicrobes.

Different scientists, supported by experiments effected with refractory animals, have advanced the hypothesis that a serum possesses curative properties because it is bactericidal. We have already remarked that experimentation upon a refractory animal can not but lead to error. In vitro experiments demonstrate that no serum taken from an immunized animal plays a bactericidal action. It is the same in vivo. The case of the anti-swine-erysipelas serum, cited as bactericidal, is particularly clear in this respect. This serum is obtained by preparing a horse by a series of injections of living cultures, but, even when the animal is thoroughly immunized, the bacilli injected are destroyed only very slowly. Several days after an injection they may be found still alive in the blood, to such a point that serum taken ten days after the last injection and used therapeutically in veterinary medicine, often contains bacilli which it is possible to cultivate. The serum is therefore not bactericidal; it is antitoxic. The bacteria cease to be pathogenic solely because their products of secretion are neutralized, and not because they are killed.

After the discovery by Pasteur of the method of vaccination by the attenuated virus, it was thought that we had in hand a general procedure. The same hope was renewed when Roux made known his results on the serotherapy of diphtheria. It was believed sufficient to prepare horses with cultures of any pathogenic bacterium whatever to provoke the appearance in the

blood of these animals of a property curative and prophylactic against the disease of which the bacterium was the agent. The results have not met this expectation. A serum enjoys a therapeutic action only if it is antitoxic, and to produce such a serum, it is necessary to inject a toxin. When a bacterium does not produce toxins *in culture* the serum obtained by the injection of the atoxic cultures has no preventive or curative property in *natural* disease. There is perhaps not a single disease for which a therapeutic serum has not been recommended during the course of the last thirty years. Unfortunately, the only ones shown to be active are those mentioned above; the others have no effect upon natural disease. Such are, for example, the anti-cholera and anti-plague sera. For the first, all that can be said is, that no matter what the dose it does not in any way modify the course of cholera.

As for anti-plague serum, it possesses a clear preventive and curative action in the experimental disease produced in rodents which are naturally susceptible, even more sensitive than man, but on the contrary, its action is entirely lacking in the human disease. Its use has, indeed, been abandoned after a number of unsuccessful attempts, both in the British possessions and in the Dutch Indies.

How do we explain these facts? We have already seen that a bacterium is able to secrete a toxin in the body although it may not do so in artificial culture medium; it all depends upon the nature of the foods, and this may be the case with the cholera vibrio. On the other hand, the secretory products of a given bacterium may enjoy a special affinity for a group of cells in one animal species and not possess, or possess to a less degree, this property for individuals of another species. But, nevertheless, this bacterium may be pathogenic for the two species. But the disease need not be identical in the two cases; in the first the antitoxic serum may possess a certain action, which is exercised not at all, or to a less degree, in the second.

It is likewise essential to consider that the disease, whatever it may be, is not provoked solely by a toxin having a specific affinity for one group of cells, but that the metabolic products of the bacterium, without an especial affinity, exercise a general action

by disturbing the colloidal equilibrium, and thus they may play an important rôle in the origin of the troubles associated with the infection. According to the causative bacterium, the first may be preponderant, the second negligible, as is the case in tetanus, while the contrary takes place in other diseases, such as typhoid fever.

It is for this reason that not all diseases are subject to serum therapy. In spite of abundant investigation, it has been impossible to prepare a serum having any influence whatever upon the course of typhoid fever or on the atoxic dysenteries, for example. This is the more marked in that an antidysentery serum, furnished by a horse which has received a series of injections of cultures, or of toxin, of the toxic dysentery strain (Shiga) does have curative properties for the dysentery caused by the toxic bacillus. The antidysentery serum furnished by a horse injected with atoxic bacilli (Flexner or Hiss) has no action whatever upon dysentery caused by these atoxic bacilli. These facts also confirm the idea, that from the point of view of preventive or curative action, the sole property of value which may be produced in the serum of an animal, is the antitoxic property.

In brief, up to the present time, the antidiphtheritic and antitetanic sera possess the most marked neutralizing properties. But it is necessary to observe that, despite their potency of action, they can do nothing for a cellular lesion already established. For this reason they should be injected at a time as close as possible to the onset of the disease, this onset being for diphtheria the first symptoms of angina, for tetanus the wound into which the spores of the tetanus bacillus may have been carried.

Has serotherapy reached its limit? This is hardly probable. d'Herelle and Le Louët have recently shown that the serum of a cow which had received a single injection of a minute quantity of a culture of the bacteriophage virulent for the bacterium of bovine septicemia, possessed marked preventive and curative power. Injected into normal cows, this serum protected against the action of several fatal doses of a culture of the virulent bacterium. It even proved of value when given several hours after the infection in spite of the rapid evolution of the disease. It was further shown that this preventive and curative power can only be due

to the presence of an antitoxin. These experiments may serve as a point of departure for a new method of preparing therapeutic sera.

Passive immunity and active immunity

The immunity developed by the injection of an antitoxic serum is established immediately, but its duration is short. It does not extend beyond twenty days in the case of man injected with the serum derived from the horse. Ehrlich has shown that the duration of this passive immunity is much longer when the serum used is derived from a species of animal the same as, or zoologically related to, the one receiving it. He has given to this transitory immunity, caused by the injection of a serum, the name of passive immunity, in opposition to the active immunity which results from the struggle of the organism against a bacterium or a toxin. Active immunity is not manifest for several days, often for several weeks, after the beginning of the infection, but its duration is often very long.

Antivenin sera

Not only against bacterial toxins have antisera been secured; antitoxin production may be considered as a general rule, a response to injections of all toxins, bacterial, animal, and vegetable. The body always responds by the formation of an antitoxin.

Ehrlich has obtained sera neutralizing the vegetable toxins, ricin, abrin, and crotin. Calmette, immunizing horses by means of cautious injections of cobra venom, has produced antivenom antitoxins. This serum is used with success in combating the intoxication following the bites of serpents. Let us observe that each species of reptile secretes a special toxin and that the action of the antitoxins is specific; it is thus necessary to inject the bitten individual with the serum of a horse immunized with the venom of a serpent belonging to the same species as the animal giving the bite. However, polyvalent sera can be prepared, useful in a given region of the world, by mixing the sera of different horses, each of which has been immunized against a venin of one of the principal species of snakes that may be encountered in that region.

Natural antitoxic immunity

During recent years it has been possible to show that antitoxic immunity explains the refractory state of human beings who resist diphtheria infection. Schick has shown that if one injects into the dermis of a susceptible individual a minute quantity of diphtheria toxin, equal to one-fiftieth of the fatal dose for a guinea-pig, it leads to the formation of an inflammatory areola followed by a small central point of necrosis. This reaction is lacking in those who are sufficiently resistant not to contract the natural disease. The method is widely employed in the United States in the prophylaxis of diphtheria for the detection of children liable to infection. This difference in susceptibility, as revealed by the Schick reaction, is due to the fact that the blood of subjects who do not react contains antitoxin which neutralizes the injected toxin and prevents, as a result, the necrotizing action. The antitoxin is present in only small quantity, but it is, however, sufficient to protect the individual from natural infection.

A diphtheria bacillus is pathogenic because it secretes a toxin which, aside from its specific action, interferes with phagocytosis, but if this bacillus is introduced into the body of an individual whose blood contains antitoxin, the toxin is neutralized, the bacillus is subject to phagocytosis, and digestion within the leucocyte takes place. We will see in effect, that it is resistance to phagocytosis which confers upon a bacterium its pathogenic character.

Park has published the following figures on susceptibility to diphtheria: of 88 infants one year old, 75 presented a negative Schick, possessing therefore antitoxin in the blood. Of these 75, 10 lost this property between the ages of two and three years, and 3 of the remaining 65 lost it between three and four years. From this, it would appear that, in round figures, 10 per cent of the children of school age in New York City are without antitoxic protection. It is, then, only these 10 per cent who ought to be subjected to prophylactic measures against diphtheria. This proportion of 90 per cent of refractory persons is increased little by little with age, so that at twenty-five years the susceptible individual is the exception. How is this natural antitoxic immunity acquired? It is not yet known.

This natural antitoxic immunity is encountered in other diseases. Brockmann, in applying the Schick test in cases of bacillary dysentery, found that in Poland about 80 per cent of men reacted out of a group of 200 individuals. He proved that the blood of those individuals which did not react possessed the property of neutralizing the toxin of the Shiga bacillus. From this it appears that in Poland 20 per cent of individuals possess dysentery antitoxin in their body fluids.

Tenbroeck and Bauer have isolated *B. tetani* from the feces of about one-third of the inhabitants of Pekin which they examined. On the other hand, they showed that tetanus is very rare in China, despite the fact that the bacilli are very widely distributed throughout the environment. Examination of the blood serum of the carriers of *B. tetani* demonstrated that such sera were high in antitoxic properties; 0.1 cc. of serum neutralizing 10, and sometimes more guinea-pig minimal lethal doses of toxin.

All of these facts show that the immunity against toxic bacteria results from the fact that the blood has antitoxic properties. But this is true only for individuals belonging to a susceptible species. In a susceptible individual immunity is of the antitoxic order. In the refractory individual it is due to the fact that the toxin is without affinity for any particular cellular type to be found within the body.

NATURE AND ORIGIN OF ANTITOXINS

Upon the question of the intimate nature and the origin of antitoxins, we are still able to advance hypotheses only. However, upon what basis other than the hypothetical does all of biology rest?

The antitoxins are probably real substances; like sensitizers the antitoxins are too stable to represent a mere state of equilibrium, as is the case with complement.

Whence are the antitoxins derived and how are they formed? Roux has advanced the hypothesis that their specificity is self-explanatory if one admits that the antitoxin is derived from the toxin. The following experiment made him reject this hypothesis. If one bleeds a thoroughly immunized animal, that is, one possessing antitoxin in its fluids, one may note that very quickly after

the bleeding the blood regains its earlier antitoxic value.   It has likewise been objected that the quantity of antitoxin formed under the action of a dose of toxin injected may be out of all proportion to the amount of the toxin, that is, that the blood of this animal is able to neutralize a quantity of toxin far greater than that which was injected.   I hardly think that these objections need be valid. The bleeding experiment simply shows that the locus of formation of the antitoxins is not in the blood, that is all.

The center of antitoxin formation is therefore cellular.   In what group of cells does it take place?   It does not appear that it can be in the nervous cells, for if one injects directly *into the brain* of a thoroughly immunized animal a minute quantity of tetanus toxin, which by *subcutaneous* injection into a normal animal would lead to no general symptoms, the animal quickly dies with symptoms of tetanus.   It is not then the cells sensitive to the action of the toxin which produce the specific antitoxin.

What is the producing cell?   The same processes of reasoning which have led us to consider the endothelial cells of the blood capillaries and of the lymphatics as being the probable locus of the formation of sensitizers, likewise lead us to consider the same cells as being the place of formation of the antitoxins.

We must admit that, in the elaborating cell, the "toxin" (I do not say toxic) micella is decomposed.   We know by experiment that the elaboration of antitoxin is slow and gradual, especially at the beginning of immunization when the cell is not yet "trained," and that, although the quantity of antitoxin formed may be capable of neutralizing a much greater quantity of toxin than that used to produce the immunization, there exists a relation between the two quantities.   If one wants greater quantities of antitoxin it is necessary to inject larger quantities of toxin.   If one stops these injections the quantity of antitoxin diminishes gradually.

In such a process I can not see how the bleeding experiment mentioned above, can show that the toxin does not enter into the constitution of the antitoxin, for if the products of the rupture of the toxin micella enter into the constitution of the antitoxin micella, it is surely certain that repeated bleedings will not exhaust the antitoxin, since the toxin having first been adsorbed or cap-

tured by the cells it should, then, slowly, enter into the formation of the antitoxins.[2]

In the case of the antitoxins, as in the case of the sensitizers (whose elaboration must be accomplished by a cellular process analogous to that of the antitoxins), and in that of the enzymes, that is to say, in the case of all substances possessing a specific action, the single satisfactory hypothesis is that which permits the antigenic substance to enter in some way into the constitution of the active substance. And moreover, may not the sensitizers and the antitoxins, be coagulating enzymes of the type of the rennets? If this is possible all is simplified, for then we may say that the cell reacts always in the same manner, by the elaboration of a coagulating enzyme.[3]

Since it is difficult for man to free himself from the finalistic point of view (every living being is a born finalist) we separate the action of the sensitizers and that of the antitoxins because the final action in the first case is the "malicious" anaphylactic reaction, while in the second case the neutralizing action is "beneficent." Fundamentally the reactional processes are exactly the same. Only the quantities of the substances reacting are different. If we inject into a guinea-pig "sensitized" by a small dose of toxin an appreciable quantity of toxin, equivalent in weight to the quantity of albumin required to cause anaphylactic shock, will we not likewise produce a true anaphylactic shock? Certainly; and since there is thus nothing to distinguish a sensitizer from an antitoxin I am forced to believe that the antigen-antibody reaction is always a reaction of coagulation.

The reactions of living matter are much less complicated than the theories would have us believe.

[2] The antitoxins are vigorously adsorbed by the serum globulins, but they are not, as many authors have thought, either the globulins themselves or properties of the globulins. This is shown particularly well by a recent experiment of Ramon, who, in dissociating the complex flocculated "toxin-antitoxin" obtained fluids poor in globulin but extremely rich in antitoxin, containing more than 60,000 units per cubic centimeter.

[3] In view of the fact that toxin manifests its action only after a period of incubation several investigators have deduced from this that it is not the toxin itself which is active, but one of the products of disintegration. This is indeed possible but has not yet been proved.

CONCLUSIONS

Endogenous immunity is twofold:

1. Natural endogenous immunity. This characterizes the refractory state of certain animal species against a given bacterium and is based upon the phagocytic reaction.

2. Acquired endogenous immunity. This may depend upon two factors, according to the nature of the causative bacterium:

a. If the bacterium is not toxic a phagocytic adaptation, always transitory, takes place, and the disease caused by such a bacterium is not immunizing. It is this transitory immunity, due to phagocytic adaptation, that is brought about sometimes (not always) by "vaccination" with killed bacterial bodies.

b. If the bacterium is toxic, the toxin causes a group of cells to elaborate an antitoxin which possesses the property of specifically coagulating this toxin. Here, there is a true acquired immunity, of greater or less duration, depending upon the nature of the toxin. Only those diseases caused by bacteria which elaborate a substance leading to a cellular reaction are immunizing in the true sense of the word.

Up to the present, all authors who have been occupied with immunity have recognized only the two preceding cases—natural immunity characterizing the refractory state, and immunity acquired as the result of a first attack. We have seen the mechanisms of these; phagocytosis in the first case, antitoxic power in the second. In both cases the defense is *endogenous;* it emanates from the body itself.

But, this endogenous immunity does not explain all. To know why an animal is naturally refractory to certain diseases, to know why certain diseases do not recur, is one thing. But by far the most interesting part of the question of the "defense" against the bacterium is thus passed by in silence. Not all susceptible animals exposed to contagion contract the disease, nor, when invaded by a pathogenic bacterium, does this animal, although still susceptible (since immunity can only become effective at the time of recovery as is demonstrated by the fact that relapses occur) always succumb. What, then, in this case, is the means of defense which allows it to go free or to recover? We

will see in the next chapter that the defense is then assured by an auxiliary, an ultramicrobe parasitic of the bacteria. The defense of the *susceptible* individual is exogenous, and this exogenous immunity is preliminary to the establishment of endogenous immunity.

CHAPTER VII

Exogenous Immunity: Bacteriophagy in Vitro[1]

BACTERIOLYSIS

Let us first of all define the term bacteriolysis, for, as we have already seen, there are few scientific terms which are more equivocal. True bacteriolysis consists in a dissolution of the bacterial body, a dissolution which can only result from a digestion, that is to say, a fermentative action. Few examples of true bacteriolysis are known. The digestive ferments, pepsin and trypsin, secreted by various glands in vertebrates, or even those of certain plants, such as papaine, have no action on living cells. Bacteria grow readily in media containing these ferments. Ferments capable of digesting living bacteria are present in leucocytes, but they are never secreted normally in the blood. As for the pretended bacteriolysins which should be present in the sera of animals having an acquired immunity, they have never existed except in theory. It has always been impossible to demonstrate their presence, for the simple reason that the action of these "lysins" results, in reality, in coagulations.

We only recognize, in fact, as a true bacteriolysis the dissolution of the pneumococcus by bile, and the dissolution of certain bacterial species by the proteolytic ferments secreted by two species of bacteria, B. subtilis and B. pyocyaneus.

The filtrate obtained by passing a culture, twenty to thirty days old, of the pyocyaneus bacillus through a porcelain filter, contains ferments able to dissolve certain living bacteria—dysentery, typhoid, cholera vibrios, among others. As in all ferment actions, the more the solution of the ferment is diluted the less is

[1] The question of the Bacteriophage has been discussed in detail in a text, one of the monographs in the collection of the Pasteur Institute, F. d'Herelle, Le Bacteriophage, son rôle dans l'Immunité, Masson, Éditeur. English trans., Williams & Wilkins Co., Baltimore, 1922. A new edition is in preparation.

the digestive power. If we add 4 to 5 cc. of a filtrate of pyocyaneus to 10 cc. of a culture of the dysentery bacillus, these bacilli are dissolved within a few hours; if we add 4 to 5 cc. of this medium after it has become limpid through dissolution of the bacilli to 10 cc. of a fresh culture of the dysentery bacilli, the action will this time be inappreciable; the bacilli will not even be killed, because of the too great dilution of the ferment.

<div align="center">SERIAL ACTIONS</div>

But we can obtain, on the contrary, what has been termed a serial action. It is only necessary to seed the culture of dysentery bacilli with living *B. pyocyaneus*. The latter develops and gradually secretes into the medium its proteolytic ferments, which dissolve the dysentery bacilli. After ten to fifteen days, the culture of the dysentery bacillus has become a pure culture of *B. pyocyaneus*. A trace of this culture inoculated into a new culture of dysentery bacilli, reproduces the same phenomenon, and this procedure can be continued indefinitely. In the case of the filtrate mentioned in the preceding paragraph, the digestive action was shown only in the first tube; in the second tube, the ferment was already too dilute to exercise its action. In the present case, on the contrary, there has been a serial action, for the pyocyaneus bacillus, the living being producing the ferment, multiplying in the first tube has dissolved the bacilli; introduced into the second it has multiplied again, and the same lytic action is reproduced; reinoculated into a third culture of the dysentery bacillus, then into a fourth, and so on, the action is continued, for the pyocyaneus bacillus, the producer of the ferment, is regenerated in each passage.

The bacteriolysis produced by the pyocyaneus bacillus is not however, of any importance from the biological point of view. In vivo, it has never been observed that this bacillus is capable of supplanting another concurrent bacterial species, for its action is too slow, fortunately, moreover, for it is not an inoffensive organism. It is the bacillus of blue pus, and has been incriminated in certain bronchopneumonias, in otitis, etc. If the action of this bacillus is dwelt upon it is because the mechanism of the bacteriolysis of which it is the agent is of extreme simplicity. Such a

reaction undoubtedly takes place and a mention of the process will facilitate comprehension of the facts which are to follow.   There, in place of a visible producer of ferments, such as *B. pyocyaneus,* we will have to do with an invisible ultramicrobe, the bacteriophage. But apart from this difference in size, the phenomena are of the same order, and the one can be comprehended as readily as the other.

THE BACTERIOPHAGE

Take 1 to 2 cc. of feces from a convalescent case of bacillary dysentery,[2] emulsify in bouillon and filter through a porcelain filter.   The filtrate, perfectly limpid, may be conserved as such during an indefinite period, therefore it is, to all appearances, sterile.   Introduce into a culture of dysentery bacilli, already developed, 4 or 5 drops of filtrate.   After a period of time, eighteen to twenty-four hours in the incubator at 37°C., it will be seen that the bouillon has completely cleared.   Microscopic examination shows that the bacilli have disappeared.   The filtrate causes, then, a bacteriolysis of the dysentery bacilli.

In a second culture of dysentery bacilli, introduce a drop of the first culture which has been clarified under the influence of the filtrate.   In a few hours the phenomenon which took place in the first tube has been repeated in the second, bacteriolysis is effected and the medium becomes limpid.   Into a third culture introduce a drop of the second after clearing; bacteriolysis is again reproduced in an intense manner, but more rapidly than in the first two, in eight to ten hours.

We are able to thus continue the series indefinitely, introducing with each passage a drop of the previously dissolved culture into a new culture well clouded with dysentery bacilli.   The dissolving action undergoes no weakening as the result of successive dilutions. On the contrary, it can be shown that the quantity of cleared culture may be reduced little by little and still yield a more and more vigorous bacteriolysis.   After a few passages a very minute quantity, a millionth of a cubic centimeter, is sufficient to bring

[2] Bacillary dysentery is taken as an example; it would be just as possible to take typhoid fever, plague, etc.

about the dissolution of all of the bacilli contained in 10 cc. of culture, and this within the space of four or five hours.

The principle which provokes the dissolution of the bacteria originated necessarily in the excreta of the convalescent. This agent has passed through the porcelain, and is found in the filtrate added to the first culture of the dysentery bacilli. After more than a thousand passages this principle is found in the last tube in greater abundance than in the original tube. It must therefore be regenerated in the course of each passage. What is the mechanism of this regeneration? This is what the following experiments will show us.

THE CORPUSCLE—BACTERIOPHAGE

Take one of the preceding cultures after complete clearing and introduce a very minute quantity of this, perhaps a drop of a ten-millionth dilution, into 10 cc. of a fairly turbid culture of dysentery bacilli. Shake, and spread a drop, perhaps a fiftieth of a cubic centimeter, on the surface of an agar slant. Incubate the tubes. After thirty minutes spread a second drop of the culture on a second agar slant; and repeat the same procedure every half-hour. After eighteen hours the culture of dysentery bacilli is limpid, as we have already shown in the preceding experiment. But here is a new fact. The surface of the agar of the first tube, where was spread the drop of the culture prior to clearing may be covered by a film of dysentery culture presenting one or two circular plaques of from 3 to 10 mm. in diameter, or the agar may be bare without a trace of culture. The agar seeded after an half-hour and after an hour has the same appearance, that is, a layer of culture spotted with one, two, or three plaques. Upon the agar seeded after one and a half hours the plaques are much more abundant, from twenty to fifty, and the tube planted after two hours has the same appearance. After two and a half hours the plaques are extremely abundant, even confluent. After four hours the agar is bare, without any evident trace of culture.

What do these plaques, apparently sterile, represent? Touch the centre of a plaque with a platinum wire and immerse the wire in a culture of dysentery bacilli. Dissolution of these dysentery

organisms results, just as though we had added a trace of the fluid from a previously dissolved culture. The dissolving principle has then multiplied on the agar, in the form of a colony.

The apparently sterile plaques, which are in reality colonies, doubtless represent points where a trace of the principle which causes lysis was deposited during the inoculation. This principle exists, therefore, in the form of particles, minute masses, for a principle in solution can hardly concentrate its action at defined points, but would rather be distributed equally throughout all the dysentery organisms contained in the culture.[3] The preceding experiment shows further that the regeneration of the active principle corresponds to a multiplication of corpuscles. The corpuscular principle which dissolves the bacteria behaves like an ultramicrobe, an ultravirus, parasitic of the bacteria, and it is to this ultravirus that the name *Bacteriophage* has been given.

CULTURES OF THE BACTERIOPHAGE

Bacteriophagous ultramicrobes inoculated into a bacterial culture develop at the expense of the bacilli; their number augments in proportion as the number of bacilli diminish. Bacteriolysis terminated, the culture of dysentery bacilli has become a culture of the ultramicrobe.

Altogether, the phenomenon is comparable to that which we have seen take place in a culture of dysentery bacilli inoculated with *B. pyocyaneus*. In the two cases the microörganism producing the lytic ferment develops, the dysentery bacilli are dissolved, and the culture of dysentery bacilli becomes a pure culture of the microörganism which produces the ferment. But *B. pyocyaneus* is an organism having an appreciable size and which renders the media turbid; the other, the bacteriophage, is infinitely smaller, and as a result of its growth it does not render the bouillon cloudy, for the latter, when lysis is ended is almost perfectly limpid.

[3] Recently Prausnitz has succeeded in determining the exact diameter of these corpuscles, as 0.02μ. They have, therefore, a diameter ten times less than that of the smallest bacteria visible under the microscope. The corpuscles are, nevertheless, much larger than the majority of the known pathogenic ultraviruses.

By artificial illumination only can a very slight clouding be perceived.

Although the dissolution of the bacteria is produced in a comparable manner in both cases, the properties of the microörganisms which cause it are very different. The pyocyaneus bacillus is not a parasite of the dysentery bacillus, the solvent action of its secretory ferments is incidental and banal. It does not require the bacterial body for its development, for it multiplies perfectly in bouillon and in the body, in the pus of wounds in particular. It is able to coexist in the intestine along with the dysentery organism. The situation differs very materially with the bacteriophage.

May one say that, in effect, the bacteriophagous ultramicrobes develop in the "bouillon" medium? No. For experiment shows that their development can not be obtained in any medium in the absence of living bacteria. The culture medium of the ultramicrobe is the bacterium and not the artificial medium. The ultramicrobe of bovine plague, for example, grows in the cow, passes from one cow to another, multiplies in a herd of cattle enclosed in a pasture; and in the same way the bacteriophage, a parasite of unicellular beings, grows in the bacteria assembled in a tube. The bacteriophage is an obligatory parasite of bacteria.

ENUMERATION OF THE ULTRAMICROBIAL BACTERIOPHAGE

Plaques once formed on agar change but little; they do not invade the bacterial covering and are never overgrown by it. Why do not the ultramicrobes invade the whole bacterial layer? Simply because the products of bacteriolysis, products resulting from the activity of the bacteriophage, interfere with its development, just as do similar products in the case of all bacteria. A bacterial colony on agar, save in rare exceptions (*B. proteus*), likewise remains localized.

Each plaque represents a colony of ultramicrobes, the issue of a single ultramicrobe deposited during the planting. It is thus possible to enumerate the ultramicrobes contained in a culture of bacilli which has become transformed into a bacteriophage culture, and this as easily as the enumeration of visible bacteria can be effected.

To fix these ideas, let us first see the technic which permits the enumeration of bacteria. Remove 0.1 cc. of a bouillon culture of dysentery bacilli and place it in 9.9 cc. of sterile bouillon. This gives a dilution of 1:100 of the original culture. After shaking, again take 0.1 cc. of this dilution and add it to a second tube with 9.9 cc. of sterile bouillon. The dilution is now 1:10,000. Repeat the same operation once more and the dilution will be 1:1,000,000. Take now, 0.1 cc. of this last dilution (or 1:10,000,000 of the original culture) and spread it carefully over the surface of an agar slant. The dysentery bacilli, very few in number because of the extreme dilution, will be deposited on the agar at points separated one from another. Each of these will multiply and form a colony, the issue of a single organism. Let us suppose that after incubation we find 17 individual colonies, meaning 17 bacilli in the ten-millionth of a cubic centimeter of the original culture. Obviously the original culture must have contained 170,000,000 bacilli per cubic centimeter.

Enumeration of the ultramicrobes is just as simple. Take a culture of dysentery bacilli which has become after clearing a culture of ultramicrobes. Repeat exactly the above procedure, except, in the place of making the successive dilutions in sterile bouillon make them in turbid cultures of the dysentery bacillus. Plant on agar 0.1 cc. of the last dilution. This last dilution contains then one ten-millionth of a cubic centimeter of the original culture of the bacteriophage, together with a considerable number of dysentery bacilli, since we have made the dilutions in well-developed cultures of this organism. The 0.1 cc. being spread upon the agar surface, the numerous dysentery bacilli will develop and give a continuous creamy bacterial layer, but the very rare ultramicrobes deposited, scattered in the midst of the dysentery organisms, will also grow. Each of them will parasitize the bacilli deposited in its proximity during the spreading and will multiply, and, the young ultramicrobes parasitizing in their turn the neighboring bacilli, the action will continue, each ultramicrobe finally forming a colony assuming the form of a circular plaque where the agar appears bare by virtue of the dissolution of the parasitized bacteria. If we have, for example, 25 plaques scattered over the culture layer, it means that 25 ultramicrobes

were deposited during the inoculation. Taking into account the degree of dilution, the original culture contained therefore, 250,000,000 ultramicrobes per cubic centimeter.

It is curious that it is possible to count ultramicrobes which are, even under the highest magnification of the microscope, totally invisible.

Many diseases of man and animals are caused by ultramicrobes. Beijerinck has advanced the hypothesis that a fluid contagium is operative. The bacteriophage gives the proof that the ultramicrobes are indeed beings possessing a mass. It may be mentioned that Beijerinck was the first to advance a conception of life quite apart from the "cellular" hypothesis.

All conditions of the experiment being alike, the number of bacteriophagous untramicrobes which develop is approximately equal; but their number will vary from one experiment to another if the conditions vary—conditions such as temperature of incubation, number of bacilli per cubic centimeter, massive or weak inoculation with the bacteriophage, etc. This number varies according to the conditions of culture from fifty millions to two or three thousand million per cubic centimeter.

The possibility of enumerating the ultramicrobes allows us to perform an experiment which, added to the preceding, gives further proof that the bacteriophage can only be an ultramicrobe. Having given, for example, a culture containing 100,000,000 ultramicrobes per cubic centimeter, a dilution containing a hundred-millionth of a cubic centimeter introduced into a bacillus culture provokes lysis. If one introduces a quantity ten times weaker into ten separate cultures of bacilli, one obtains, on the average, bacteriolysis in but one of the ten cultures. The other nine show no change, giving normal bacillus cultures and the subcultures from them are normal. This experiment is additional proof that the bacteriophage is composed of particles, of which only a single one is liable to effect lysis, but if this particle is lacking, no change, however weak, is observed in the bacterial culture. The lytic principle is then represented by a particle which multiplies; and an invisible particle which multiplies can only be an ultramicrobe.

THE BACTERIOPHAGE: AN INTERNAL PARASITE

The bacteriophage multiplies only at the expense of living and normal bacteria. Like all living beings the bacteriophage is inhibited in its action by the products which result from its activity. Bacteriolysis ceases to be complete when the number of bacteria in the medium is above a certain concentration—for a very active strain of bacteriophage about 500,000,000 bacilli per cubic centimeter. In more heavily charged media lysis takes place but the medium remains somewhat cloudy, the more so as the number of bacteria is the greater. However, transfers from such cultures remain sterile; the bacteria are killed.

Direct experiment and ultramicroscopic observation show that the bacteriophage can only be an internal parasite.

It may be recalled from the second experiment cited that the multiplication of the ultramicrobes seemed to take place intermittently, in successive jumps. The interval of time separating each liberation of ultramicrobes into the medium varies, according to the strain, between one and three hours, at a temperature of 37°C. On the other hand, if one inoculates a strain of the bacteriophage into a series of cultures of dysentery bacilli and centrifuges one of these cultures every quarter of an hour, it is to be observed that after fifteen minutes the ultramicrobes have disappeared almost entirely from the fluid. They are carried down by gravity along with the bacteria in the sediment at the bottom of the tube (this does not take place in the control tubes where the ultramicrobes are inoculated into a bacterial culture for which the bacteriophage shows no action). They reappear in the liquid in from one to three hours afterward, and in numbers 15 to 25 times greater than were the original ultramicrobes inoculated. Their number then diminishes, then a new liberation occurs after another lapse of one and a half hours. The growth of the ultramicrobes continues thus by successive liberations.

Examination with the ultramicroscope offers an explanation for this phenomenon. If one follows a bacillus culture inoculated with the ultramicrobe one sees that a certain number of the bacteria become deformed, first becoming swollen, then taking a spherical form, and at the same time very fine brilliant points appear in the interior of the bacilli. Then, suddenly, there is

a rupture of the rounded bacillus and there remains in its place a slight cloud of protoplasm in which are embedded the brilliant particles. The protoplasm dissolves within a few minutes liberating the corpuscles into the medium.

It appears from these facts that the bacteriophagous ultramicrobe penetrates the interior of a bacterium, multiplies there, secreting lytic ferments, and forming a colony of 15 to 25 individuals. Then the bacterium ruptures, liberating thus the young ultramicrobes, each of them proceeding to parasitize a new bacterium. At 37°C. from one to three hours elapse between the moment when the bacterium is parasitized and the time of its rupture.

THE VIRULENCE OF THE BACTERIOPHAGE

We have taken as an example the case of the bacteriophage active against the dysentery bacillus, but many species of bacteria undergo bacteriolysis under its influence—typhoid and paratyphoid bacilli, *B. coli*, *B. gallinarum*, *B. proteus*, the staphylococcus, *B. pestis*, the bacteria of the hemorrhagic septicemias, etc.

In view of the variety of bacterial species which have been seen thus far to be susceptible to the action of the bacteriophage it appears that it must be a general fact, —that all bacterial species are susceptible of being parasitized.

In all the preceding discussion we have considered the case of the extremely virulent bacteriophage, capable of causing a complete clarification of a bacterial suspension. Upon removal from the body, different strains of the bacteriophage vary markedly; it may have an activity hardly detectable, or one causing the most violent lysis. Experiment proves that this difference of action is not due to the number of bacteriophages but that it is to be ascribed to the activities of the particular strains involved. There is, then, a variable virulence, the term virulence being taken in its proper sense of "ability to vegetate in vivo." Unless this virulence is extremely weak it is always possible to exalt it by serial passages with a sensitive bacterium. We will return to this fact in a moment.

BACTERIAL IMMUNITY

Even in the case of a complete lysis of a bacterial suspension it is often observed that a medium, after remaining limpid for a number of days, slowly clouds again. The mechanism of this return to growth is interesting to consider.

Inoculate a quantity, perhaps 250 cc., of a culture of dysentery bacilli with one drop of a culture of a very virulent strain of the bacteriophage. After a few hours the medium is limpid, since the bacilli are dissolved. At this time divide the 250 cc. into 25 tubes, each containing 10 cc. After two, three or four days, we will often see one or two of these 25 tubes become turbid, the others remaining limpid indefinitely. It must necessarily be admitted that among the 60 billion or so of bacilli contained in the 250 cc. of culture one, two, or three have been able to resist parasitism with the extremely virulent ultramicrobe. These bacteria have acquired a resistance, an immunity, and have been able to develop despite the ultramicrobes. One has then a secondary culture of the bacterium which coexists in the medium with the virulent bacteriophage, and experiment shows that these bacteria have acquired a resistance to the action of the bacteriophage. It is, moreover, possible to separate these bacteria from the ultramicrobes, when it can be shown that their immunity is lost little by little in successive cultures.

The acquisition of immunity is not the property of superior organisms only, for the bacteria are equally capable of acquiring it, and it should be noted that the less virulent the strain of bacteriophage used the greater the number of bacteria which develop immunity.

If, instead of isolating the bacterium, we inoculate, en bloc, this secondary culture, we obtain mixed cultures, indefinitely cultivable in series, composed of resistant bacteria and virulent ultramicrobes.

For a long time bacteriologists have described bacterial mutations, but they have not known the cause of the phenomenon. These mutations are produced always under the influence of the bacteriophage; a pure bacterial strain is not subject to transformations.

We again meet with a general fact; a mutation is never produced without a determining cause, and this cause is represented, either by a variation in the conditions of the environment or, and especially, by parasitism. The phenomena of bacterial mutation under the influence of parasitism by the bacteriophage are absolutely of the same order as those described by Noël Bernard for plants.

In what, then, does this mutation consist? The bacterium does not remain passive before the attack of the bacteriophage. It defends itself, and in two different manners. It produces a mucoid capsule which may assume such a development that the colony on agar of such a bacterium presents a zoögleic appearance. This passive defense is accompanied by an active one, the bacterium secreting products which inhibit the action of the bacteriophage. In brief, the defense of the bacterium against the bacteriophage is the same as the defense against the phagocyte. Moreover, experiment shows that a bacterium which has acquired resistance to the bacteriophage becomes by this fact resistant to phagocytosis.

The possibility of the bacterium developing a resistance is a phenomenon of extreme importance in biology, as we will shortly see.

INCREASE IN THE VIRULENCE OF THE BACTERIOPHAGE

The phenomena in which the bacteriophage takes part are still further complicated by the fact that the virulence of a given strain of bacteriophage is not directed solely toward a single bacterial species, but manifests itself at one and the same time with variable intensity, upon the bacteria of related species and sometimes upon unrelated forms.

It is always possible to exalt the virulence of a strain of the bacteriophage against a bacterium for which it manifests only a feeble activity. It suffices to cultivate repeatedly this bacteriophage with the bacterium against which a higher virulence is desired.

This method of exaltation of virulence by successive passages with the susceptible bacterium is in all respects comparable to the method of Pasteur for the increase in virulence of a bacterium by repeated passages through a susceptible animal.

We may wish for example, to exalt the virulence of a strain of the bacillus of swine erysipelas for the rabbit. We inoculate a small quantity of a culture of this bacillus into a rabbit. When this animal dies we remove a small amount of the blood and inoculate that into a second rabbit, and after its death we again withdraw some blood, and inoculate a third rabbit, and so on. At the end of a certain number of passages, if the minimal fatal dose of the original culture was, for example, 0.001 cc. it may require now only a thousandth part of this amount to kill a rabbit. And it is to be observed that in proportion as the virulence increases for the rabbit it is attenuated for the pig.

It is exactly the same for the bacteriophage. Take a strain possessing a high virulence for the dysentery bacillus, and a weak action on typhoid bacilli. Inoculate two or three drops of a culture of such a strain of bacteriophage into a culture of the typhoid bacillus. There is no clearing of the medium; only a few plaques on agar show us that this strain possesses a certain activity for this bacillus. After twenty-four hours filter through a candle to remove the bacilli which have acquired a resistance to the ultramicrobes which have developed. Cause these ultramicrobes which have passed through the filter and are found in the filtrate to act upon a new culture (*fresh*) of typhoid bacilli; that is, on typhoid bacilli without an acquired resistance. This time a larger number of bacilli are susceptible to being parasitized, for the more virulent of the inoculated ultramicrobes have grown already at the expense of the typhoid bacilli in the first tube. A selection takes place. After twenty-four hours, filter and make the filtrate act upon a third fresh culture of typhoid bacilli. This time we may obtain a clearing of the medium. Continuing these passages, always in the same manner, we obtain finally ultramicrobes of maximum virulence for the typhoid bacillus, that is to say, they quickly cause a total lysis of the culture. At this time we can usually demonstrate that the virulence for the dysentery bacillus is considerably reduced.

The phenomenon of the exaltation of virulence of the bacteriophage for a given bacterium is a duplication, in the most minute details, of that of the increase in virulence of a bacterium for a given animal. The ultramicrobe and the pathogenic bacterium

on one side, the susceptible bacterium and the susceptible animal on the other, react in an identical manner. This is not astonishing for in both cases there is a parasite which attacks on one side, and a being which defends on the other. The mechanisms of attack, like those of defense, are the same for all living beings.

NATURAL AND ACQUIRED IMMUNITY OF THE BACTERIUM FOR THE BACTERIOPHAGE

The behavior of different bacterial species toward the bacteriophage is not identical for all of them. For certain species, *B. dysenteriae*, *B. pestis*, when a strain undergoes bacteriophagy by a given bacteriophage all other strains are susceptible. The virulence of a single bacteriophage may be different for the several strains of the bacterial species, but by passages it is always possible to exalt it. These bacterial strains are therefore "homogeneous" with reference to the bacteriophage. Toward other bacterial types, *B. coli*, *B. typhosus*, and the staphylococcus, the the situation is otherwise. Here a bacteriophage may attack a certain number of strains of a bacterial species and fail to have any action upon others, which, on the other hand, may be lysed perfectly by other strains of bacteriophage.

Certain bacteriophage strains may enjoy an absolute specificity. Such a specificity is acquired by a bacteriophage after a very long series of passages exclusively at the expense of a given bacterium. On the other hand, other bacteriophage strains can be isolated with which the action extends to all, or nearly all, bacterial strains. Between the two extremes all variations of extended virulence are encountered.

It is quite essential to remember this fact, for some authors have confused this natural resistance, this natural immunity of certain bacteria for a given strain of bacteriophage with a resistance acquired by a bacterium which has been in contact with the bacteriophage and which has resisted it. The first is natural, and is not lost. The second is lost gradually by a series of pure cultures out of contact with the bacteriophage. The phenomena of immunity in the bacteria are strictly comparable with those observed in higher animals. Nature is less complicated than we would make it by our theories which fail to take cognizance of the facts.

CHARACTERS OF THE BACTERIOPHAGE

We have seen that many bacterial species are susceptible to the action of the bacteriophage. The fact that a single strain of bacteriophage is active at the same time against different bacterial species shows that there is in all cases but a single species, capable of being virulent for any bacterial species whatever. This action of a given bacteriophage manifests itself upon species as distantly related as *B. coli* and *Staphylococcus aureus*, or *B. coli* and *B. pestis*. I had a strain of bacteriophage, isolated from the intestinal tract of a dysentery patient, which, after several hundred passages in vitro, always with *B. dysenteriae*, still possessed a certain activity for several bacterial species. In my laboratory Schurman determined its activity, by observation of the characteristic plaques, upon several different strains of *B. coli*, *B. typhosus*, the paratyphoids A and B, the bacillus of Gaertner, two types of pasteurella, one race of *B. proteus*, *Vibrio metchnikovii*, and upon all strains of *B. dysenteriae*, Shiga, Flexner and Hiss. By repeated passages with any one of these bacteria he was able to increase the virulence for this bacterium and to obtain lysis in a liquid medium.

The complement fixation reaction confirms the unicity of the bacteriophage. Whatever may be the bacterial species attacked, the bacteriophage possesses always the same antigenic properties. It is then, but a single organism.

If one prepares a rabbit by injections of a culture of the bacteriophage which had developed at the expense of dysentery bacilli the serum of the rabbit will contain an amboceptor which fixes not only with the ultramicrobes developed at the expense of the dysentery bacillus, but equally well with those which have developed at the expense of typhoid bacilli, the staphylococcus, *B. pestis*, etc. This proves indeed that in all cases the ultramicrobes belong to but a single species.

The substance of the bacteriophage is of protein nature for it possesses antigenic properties, and we have seen that this is a function of proteins only. Furthermore, Wollman has shown that the bacteriophage is digested by trypsin.

The resistance of the bacteriophage to the different agents of destruction is almost the same as that of the spore-forming bac-

teria. It is lower than that of certain of the other ultramicrobes, that of the tobacco mosaic, among others. The bacteriophage loses its activity upon being held at a temperature of 68 to 75°C. for thirty minutes. It is peculiarly sensitive to certain substances which have no effect upon the ferments. It is killed by contact for a week with 95 per cent glycerine (Bablet) and in a few hours by pure glycerine (Proca), after forty-eight hours in 90 per cent alcohol, after a few hours in a 1 per cent solution of a neutral salt of quinine (Eliava and Pozerski), and after eight days in 70 per cent acetone. It is more sensitive to free acidity or alkalinity than the majority of bacteria. In liquid media it remains alive for more than five years, but its virulence diminishes, although very slowly. When dry it does not remain alive for more than a few weeks.

The bacteriophage is sensitive to the oligodynamic action of metals (da Costa Cruz). When suspended in a fluid containing a sheet of silver it is quickly destroyed, a peculiarity which it shares, however, with other microscopic and ultramicroscopic beings.

It is not possible to discuss at length the extremely interesting nature of the action of antiseptics upon the bacteriophage. But, in brief, this study shows that the bacteriophage appears to exist both as a vegetative form and as a resistant form. The latter seems to offer a resistance comparable to that of bacterial spores, while the vegetative form is extremely sensitive and is destroyed by quantities of antiseptics so weak as to permit the normal growth of bacteria (*B. coli*, for example).

THE LIVING NATURE OF THE BACTERIOPHAGE

All of the experiments dealing with the bacteriophage have been recognized as correct by all authors who have undertaken to verify them. Only the conception touching the nature of the active principle has been subject to discussion. These controversies are of a character too technical to be elaborated here, and it will be sufficient to say that some bacteriologists have desired to show that the principle which causes transmissible bacteriolysis is derived from the bacterium itself which is subjected to the lysis. It must be, then, a true phenomenon of suicide in perpetual series!

None of the authors, partisans of this hypothesis, have attempted to provide any explanation whatsoever concerning the intimate nature of such a phenomenon. Indeed, none of them have tried to reconcile this hypothesis with the facts!

Doubtless these discussions will appear strange within a few years for they are in fact but a renewal of the old ideas of Stahl, the father of phlogiston, concerning fermentation. These ideas were restated by Liebig in his discussion with Pasteur, and it has been supposed that they had finally disappeared from science, but it is not true as we see.

The greatest error of the partisans of the theory of autolysins consists in a faulty logic. The intimate nature of any living being whatever can not be determined by the results of indirect experiments bearing on time or place, that is to say, that the locations in which it may be encountered or the circumstances in which its presence may be demonstrated can not in any case have any significance in establishing its intimate nature.

Recently this discussion has widened; various scientists, Doerr among others, facing the fact shown by their experiments, that the bacteriophage possesses all of the characteristics, all of the peculiarities of other ultraviruses, have questioned the living nature of all ultraviruses, the agents of the contagious diseases which we will consider in a following chapter.

But, all of the viruses known are parasites, and no one has yet succeeded in cultivating them in the absence of the cell which they parasitize. It can be understood then that experimental investigation upon such beings must be extremely difficult to accomplish, except with a single one, the bacteriophage, and this is excepted only because it is a parasite of organisms which can themselves be readily cultivated. If one introduces the bacteriophagous ultravirus into a culture containing some billions of susceptible bacteria one causes an epizoötic which destroys the bacteria. This epizoötic is bacteriophagy in vitro. The phenomenon is exactly the same if one introduces the ultravirus of avian plague, for example, into a district where there are many individuals of the susceptible species, the chicken. In both cases there is an epizoötic, but in the first the action is relatively simple, since, all of the phases taking place in vitro, the phenomenon is relatively easy to study,

and the nature of the actions and reactions of the two beings involved can be much more readily determined.

Because of the behavior of the ultramicroscopic pathogenic agents, their nature is directly determinable only in the case of the bacteriophage. Obviously, the determination of the living nature of the bacteriophage is, strictly speaking, valid for this being alone, but it is none the less true that such a fact, once established, makes it highly probable that all ultraviruses presenting comparable characteristics and acting under similar conditions are of the same nature.

How can we say that an organism is a living being? By virtue of the fact that it exercises serial actions? Evidently not. For in this case fire would be living, and in autocatalytic reactions, the autocatalyzer ought also to be living, an assumption which is manifestly false. Is it because it has the power of motion? No, for the clouds would be alive. Because it multiplies? Hardly, for the colloidal micella multiplies, to a very rudimentary degree it is true, but this property of multiplication confers upon the micella some very peculiar attributes which, although the micella is not alive makes it something far from inert. Life is not a metaphysical property, and it is precisely because of this that a limit can not be drawn between inert matter and living matter. There is an insensible transition between inert and living matter, and if the ultraviruses come into consideration in this discussion it is simply because they constitute without doubt the lowest stage of living matter.

We do not know what life is. We declare that a being is alive if it possesses a group of properties, considered, by definition, as appertaining to living beings alone. Does the being with which we are concerned possess, or does it not possess, these criteria of life? That is the real question. If it possesses them, there is no room for doubt. Indirect experiment can not modify the conclusion. But there are experiments, on the contrary, which must be interpreted in accordance with our knowledge of the subject, acquired by the observation of facts.

We have considered from the beginning of this text the characters which appertain to living beings as such, characters which can not belong to anything else, the criteria of life, such as the prop-

erties of assimilation and of adaptation, with their inevitable corollaries, the capacity to multiply and to present variability.

Does the bacteriophage possess these criteria, in spite of its absolute invisibility? Invisibility is not an obstacle, for one is not able to see assimilation or adaptation. These are properties. We can observe their effects, and such observation permits us to deduce that these properties are an appanage of the being under consideration. Hence, the bacteriophage does not have to be visible in order to determine its nature.

All authors actually admit, and experiment is conclusive in this respect, that the bacteriophage exists in the form of corpuscles and that these corpuscles multiply during the course of the action.

If it were a visible bacterium the faculty of multiplication would be considered as a proof of assimilation, and from this one would conclude that he was dealing with a living being. In the case of the bacteriophage, however, the question is complicated by the possible objection that the lytic principle which is regenerated may be a product of bacterial metabolism.

A priori this objection is perfectly valid. Consequently it becomes necessary to first demonstrate that the bacteriophage is an *autonomous* corpuscle, independent of the bacterium subjected to its action.

AUTONOMY OF THE BACTERIOPHAGE

1. In 1916 I isolated a strain of bacteriophage active at the time of isolation for *B. dysenteriae* Shiga, *B. coli*, and *B. typhosus*. After 1200 passages effected solely at the expense of *B. dysenteriae* the ability to attack *B. coli* and *B. typhosus* had persisted. But, the virulence of the bacteriophage for *B. dysenteriae* does not necessarily involve a virulence for *B. typhosus*, for a great many strains of the bacteriophage may be found which are active for but one of these two bacteria, and are devoid of all activity for the other. Since this property is not constant, we must admit that it is a property of the initial principle which multiplies and that this principle is independent of the bacterium at the expense of which it multiplies, since after more than 1200 passages at the expense of *B. dysenteriae*, the power of attacking *B. typhosus* had persisted.

2. I have isolated some hundreds of bacteriophages and I have never encountered two which possessed exactly the same virulences. But, to accomplish the isolation of the bacteriophage, I have constantly employed the same bacterial strains, the same strain of *B. dysenteriae*, the same strain of *B. typhosus*, etc. If the bacteriophage were a bacterial product, it is clearly evident that all of the bacteriophages isolated active for *B. dysenteriae* should possess the same properties, since being cultivated at the expense of the same dysentery bacillus, all have regenerated necessarily at the expense of the same bacterium. But this does not happen. Every strain of bacteriophage isolated has presented its own properties. This again appears to be a proof that it is an autonomous being, in which the characters do not depend upon the bacterium at the expense of which it regenerates.

3. I have indicated, and all authors are actually in agreement on this point, that each strain of bacteriophage possesses individual characteristics, chiefly with reference to the extent of the virulence for different bacterial species. In addition, for *B. typhosus*, the staphylococcus, *B. coli*, etc., certain strains of bacteriophage attack a certain number of bacterial races and remain without action upon others. The following experiments of Janzen and Wolff are based upon this character. They took three bacteriophages, possessing different properties: Bacteriophage I leading to bacteriophagy of strains A, B, D, and E of *B. typhosus*, Bacteriophage II effecting bacteriophagy with strains A, C, and E, and Bacteriophage III, causing the lysis of strains A, B, and F.

Each of these three bacteriophages were subjected to a large number of passages, and in every instance, against strain A of *B. typhosus*. If the bacteriophage is a product of the metabolism of the bacterium it is certain that the three bacteriophages should become identical, since all three were regenerated at the expense of the same bacterium. If, on the contrary, the bacteriophage is an autonomous being, a parasite of the bacterium, developing at its expense and transforming bacterial substance into bacteriophage substance, it will possess its own properties and will conserve them whatever may be the bacterium at the expense of which it develops. And this second alternative is exactly what happens. After many passages at the expense of the A strain of *B. typhosus*, Bacterio-

phage I attacked only strains A, B, D, and E, as before, Bacterio-
phage II, attacked only A, C, and E, and Bacteriophage III was
active for strains A, B, and F. "Thus," conclude Janzen and
Wolff, "we are in perfect accord with d'Herelle, the bacteriophage
can only be a living autonomous being, independent of the bac-
terium which is subjected to its action."

4. Maitland has given a proof of the same nature, basing his
work upon the activities of bacteriophages for *B. dysenteriae* and
B. paratyphosus B. His conclusions are analogous to those of the
Dutch authors.

5. Gratia experimented with a bacteriophage *v*, active solely
for *Staphylococcus albus V*, and with a bacteriophage *h* which was
polyvalent, attacking both *Staphylococcus albus* and *Staphylo-
coccus aureus*. He effected serial cultivations of the polyvalent
bacteriophage *h* at the expense of Staphylococcus *V*. If the
bacteriophage is a product of bacterial metabolism it would appear
that the monovalent character of bacteriophage *v* derived its
properties from *Staphylococcus albus V* at the expense of which it
regenerated, and from the time when we cultivate the polyvalent
bacteriophage *h* at the expense of this *Staphylococcus albus* it
should lose its character of polyvalency and become monovalent.
But, after a long series of regenerations at the expense of Staphy-
lococcus *V*, the bacteriophage *h* retained intact its polyvalent
nature. This indicates that this property is characteristic and is
independent of the properties of the bacterium at the expense of
which it develops. The bacteriophage is therefore an autonomous
being.

6. Prausnitz has given another proof of the autonomy of the
bacteriophage. This is based upon its antigenic properties.

7. Asheshov has likewise furnished proofs of autonomy based
upon crossed experiments with a bacteriophage which produces
small plaques and another which produces large plaques. This
property—the formation of small or large plaques—is in certain
cases an inherent individual character of the strain of bac-
teriophage.

These experimental observations, many in number and varied
in the particular features which they emphasize, show clearly
through the differing properties of individual strains of the

bacteriophage, that the principle in question is an autonomous living being, independent of the bacterium.

The proof of autonomy predicates the power of assimilation, since the independent corpuscle multiplies indefinitely at the expense of the bacterium. There is necessarily a transformation of bacterial substance into bacteriophage substance. In other words, just as surely as the bacteriophage is autonomous, that is, absolutely independent of the bacterium, and just so surely as it multiplies at the expense of the bacterium, then it can only be a parasite of this bacterium.

ADAPTATION OF THE BACTERIOPHAGE

All authors admit that the virulence of the bacteriophage may increase for a given bacterium, or that it may diminish, according to the conditions of the moment. This is then a phenomenon of adaptation analogous to that observed with all parasites.

The fact of attenuation and of exaltation of virulence is sufficient by itself to show that the bacteriophage is an autonomous parasite. Certain authors (Seiffert) while admitting the fact, have tried to maintain that it is not the bacteriophage which adapts itself, but rather the bacterium. An obvious reply would be that it is not the bacterium with which the passages are made, since each passage involves the action of the filtrate of a preceding lysed culture upon a fresh normal suspension of bacteria. By virtue of the fact that only the filtrate is concerned in the passages the adaptation must be of something which is found in the filtrate.

But this is not all. It is certain that the bacterium, which is also a living being, must react, must likewise undergo adaptation. Constant experience shows that this is just what happens, but the adaptation which takes place, far from tending toward a destructive action, as would be the case if the bacterium adapted itself to the secretion of a lytic substance, reacts against the bacteriophage by a process of adaptation tending to hinder the action of the bacteriophage. The bacterium acquires a resistance. This resistance may, indeed, reach to a completely refractory condition, and, in such a case, it is the bacterium which destroys the bacteriophage (d'Herelle, Flu).

The bacteriophage adapts itself to a more and more vigorous attack against the bacterium, and the bacterium accustoms itself to resist this attack. Considering only experimental facts this is clearly evident when no pretense is made to interpret these facts to make them fit into a preconceived theoretical scheme.

But there are still other points. The bacteriophage adapts itself to harmful effects of the medium. I have shown that the bacteriophage can gradually adapt itself to the harmful action of glycerol and of acids. Asheshov has habituated a bacteriophage, originally unable to effect bacteriophagy in an acid medium, to act very strongly after a number of passages in a medium of increasing acidity. Wolff and Janzen have succeeded in adapting it to different antiseptics.

We have already seen that the bacteriophage functions as an antigen and that the serum of an animal which has received serial injections of a bacteriophage possesses the property of inhibiting bacteriophagous action. Prausnitz has shown further that it is possible to adapt the bacteriophage to resist the inhibiting action of an antiserum. Once this adaptation is accomplished bacteriophagy takes place in any quantity of antiserum, although prior to the adaptation, an amount of a thousandth of a cubic centimeter or even less paralyzed bacteriophagy completely.

The proofs are then multiple: The bacteriophage possesses the power of adaptation. We have seen that it also possesses that of assimilation. It possesses likewise the two corollaries of these powers; the faculties of multiplication and variability as everyone admits.

THE LIVING NATURE OF THE BACTERIOPHAGE

Possessing the criteria of life the bacteriophage is therefore of necessity living. This is not solely hypothesis, it is an absolute certainty, for nothing can possess the criteria of life unless it is a living being.

Prior to such proof, suppose, although it is not true, that the bacteriophage possessed a character which appeared to be incompatible with the idea of life. From the time when it possessed *all* of the characters of living beings it would be necessary to accept it and to recognize that life is possible under conditions which

up to that time had been considered impossible. This would not have been the first time in the history of science that theory had been overturned, but, I repeat, in the case of the bacteriophage we are not confronted by such a condition. None of its characters, none of its properties, are out of accord with the properties and the characters of living beings. Furthermore, it is impossible to interpret these properties in the abstract sense apart from life.

In a word, the bacteriophage, possessing the powers of assimilation and of adaptation, the faculties of reproduction and of variation, is of necessity a living being.

This fact is of fundamental importance in biology, for it shows that a being, which can be constituted only of a simple protoplasmic micella possesses all of the properties of living beings, bringing proof that life is not the result of a cellular organization but of a special physico-chemical constitution of a micella.

CONCLUSIONS

The phenomenon of bacteriophagy in vitro can be summarized thus. A principle existing in the intestinal tract can bring about the dissolution, the lysis, of living bacteria. This lysis is a serial action. At the same time that the bacterial dissolution takes place the lytic principle regenerates. The dissolution ended, that which was a culture of bacteria has become a culture of the lytic principle. A trace of this culture, inoculated into a new culture of the bacterium provokes the same phenomenon of dissolution, with regeneration of the lytic agent. Bacteriophagy can thus be continued indefinitely.

The lytic principle occurs in the form of ultramicroscopic corpuscles which pass through ultrafilters. Despite their smallness (diameter, about 20 millimicrons) it is possible to enumerate them, since upon solid media isolated colonies of the corpuscular bacteriophage are to be found.

The bacteriophagous corpuscles are endowed with the powers of assimilation and adaptation, the faculties of multiplication and of variation. They are thus necessarily living beings since they possess all of the characteristics of other living beings.

A single bacteriophage is usually virulent, at the same time, for a certain number of bacterial species. This virulence is variable

and is subject to increase or attenuation. Increase may always be secured in vitro by the method of passages at the expense of the bacterium for which it is desired to increase the virulence.

The bacterium does not remain passive before the attack of the bacteriophage. It is capable of resistance. It is even able, when the conditions for it are favorable, to acquire a complete immunity.

The phenomenon of bacteriophagy is very complex. The dissolution of the bacteria, which is the visible manifestation, is the resultant of the action of the bacteriophage, the bacterial parasite, and the reaction of these bacteria.

But, since this bacteriophage, parasitic in the bacteria, is found in the intestinal tract, can this phenomenon of bacteriophagy which we have seen taking place in the test-tube take place in the same way in vivo?

Exogenous Immunity: Bacteriophagy in Vivo

VIRULENCE OF THE BACTERIOPHAGE IN VIVO

First of all it is essential to devise a method of evaluating the virulence which a bacteriophage has for a given bacterium in vivo at a given moment.

Without entering into a discussion of the details of the technic we may say that a bacteriophage possesses a low virulence $(+)$ when it fails to provoke macroscopic lysis of a bacterial suspension in bouillon and does not inhibit the growth in a fluid medium weakly inoculated with the experimental bacterium. Its presence can only be detected by the development of rare colonies (plaques) on agar seeded with a bacterial suspension to which has been added 1 part in 10 of the homologous bacteriophagic fluid.

The virulence may be termed average $(++)$ when, on being added to a bacterial suspension, the bacteriophage does not cause an appreciable lysis, but stops temporarily, for a time sufficiently long to be noted in comparison with the control, the growth in a fluid medium seeded with the test bacterium. When spread upon agar, a suspension of the test organism containing one-tenth its volume of the bacteriophage-containing fluid shows many bacteriophage colonies (plaques).

The virulence is high $(+++)$ when a suspension containing 250 million bacteria per cubic centimeter undergoes complete lysis or an almost complete lysis when the bacteriophagous fluid is added to a concentration of 1 per cent, but when secondary cultures constantly develop.

Maximum virulence $(++++)$ is when the lysis of a suspension containing 250 million bacteria per cubic centimeter is complete under the influence of the bacteriophagous liquid in a concentration of 1 part in 1000, and when this lysis is permanent, with but few exceptions (not more than 1 tube in 10).

We know that it is always possible to enhance the virulence of a weak bacteriophage by the method of passages, that is, by adapting the bacteriophage for a given bacterium. But here, in measuring the virulence which it possesses in the body of the individual from whom it is derived, that which we must determine is not its possibilities for virulence, but the virulence which it actually possesses at the moment of its isolation. The degrees of virulence which are indicated in the discussion in this chapter refer to the condition of bacteriophage as recovered directly in the filtrate prepared from the feces of the individual under examination, or of the bacteriophage from the blood, as the case may be.

THE BACTERIOPHAGE IN THE NORMAL INDIVIDUAL

Let us begin by considering the bacteriophage within the normal individual, in persons who are apparently healthy.

Throughout the year 1918 I examined every fifteen days the feces of a normal man, searching for the presence of a bacteriophage active for *B. coli*, and at times for other bacteria. Seventeen times I found a bacteriophage active for *B. coli*. Eight times it was also active for one or another of the bacteria of the colon-typhoid-dysentery group. Six examinations were negative. Was the bacteriophage absent?

To conclude that the bacteriophage is certainly absent from the intestinal contents it would obviously be necessary to test the filtrate from the feces against *all* susceptible bacteria which might be found in the intestinal tract. This is practically impossible, because of the enormous possible number of bacteria. Although this difficulty might be surmountable, it is sufficient to make the tests upon about 100 bacteria, which is tedious but still possible. This is what I attempted with the 6 filtrates which did not appear to contain a bacteriophage active for *B. coli*. One cc. of these filtrates was introduced into suspensions of all of the intestinal bacteria to be found in the culture collection at the Pasteur Institute. One of these 6 filtrates contained a bacteriophage strongly active for *B. paratyphosus B* ($+++$); a second contained a bacteriophage of moderate activity ($++$) for *B. enteritidis;* a third a strongly active ($+++$) bacteriophage for a salmonella (*B. suipestifer*).

There remain, therefore, three examinations in which the presence of a bacteriophage was not demonstrated. But, to the source of error which I have indicated (the practical impossibility of testing the filtrate upon all bacterial species liable to be found in the intestinal tract—and, in fact, if one reflects, upon all bacteria with but few exceptions) must be added another still greater. We have seen that, in relation to the bacteriophage there are both homogeneous and heterogeneous bacterial species. Among the last is to be found *B. coli*, which is the most heterogeneous of all of the bacterial species. To affirm that a filtrate of feces does not contain a bacteriophage virulent for *B. coli* would require the testing of this filtrate against all strains of *B. coli*. Here we have not only a material difficulty but an absolute impossiblity. I wished to determine if these deductions were correct. From a bouillon dilution of 5 cc. of the feces of a normal man I prepared 100 cc. of filtrate. From the same fecal material I also isolated 50 colonies presenting the aspect of *B. coli*, formed of bacilli morphologically *B. coli* and yielding indol. The filtrate did not contain a bacteriophage which was virulent for any of the 5 strains of the laboratory collection. Against the 50 strains isolated from the intestine at the same time, and all transplanted on agar twelve times to diminish their resistance in case they had any, 9 were sensitive to the action of the bacteriophage isolated (1 +++, 3 ++, and 5 +). Furthermore, experiments with crossed bacteriophagy showed that the filtrate contained not one, but at least 4 different strains of the bacteriophage. (Three attacked electively each one colony of *B. coli* (+++, ++, +); the fourth attacked 6, its virulence being moderate (++) for two colonies, low (+) for four others).

These experiments show that whenever the bacteriophage appears to be absent from the feces of a normal individual, it is only necessary to continue the investigation to disclose it. It is simply a question of technic.

As a matter of fact, I have conducted many examinations of the feces of normal men, both in France and in Indo-China, and the conclusion is that, with very rare exceptions (referable as I have shown to an inadequate technic) one can find in the intestinal tract of man a bacteriophage active for *B. coli*. And this activity

often extends to other members of the colon-typhoid-dysentery group. In healthy man the virulence is ordinarily weak.

I have examined the feces of different animals, including 62 horses, 46 cattle, 48 buffaloes, 6 swine, 2 goats, 2 rabbits, 2 cats, 1 monkey, 70 chickens, 2 geese, several silk-worms, and several locusts, all apparently normal. In every case, without exception, I have found a bacteriophage virulent for one or another bacterial species of the colon-typhoid-dysentery group, generally for several at once, one of which was *B. coli*.

In the healthy animal the bacteriophage is usually of much higher virulence than in normal man, as might be suspected, for the mode of life of the animal is more exposed to accidental contamination by varied bacteria. Whether these bacteria may be pathogenic for the animal is of little importance, the pathogenic character having nothing to do with bacteriophagy.

Various authors have reported similar experiments. The most extended are those of Tomaselli, who has investigated the feces of man and of horses. This author has shown the presence, in all cases, of a bacteriophage active for one or another of the representatives of the colon-typhoid-dysentery group. A virulence for *B. coli* was by far the most frequent finding.

We may conclude from these experiments that the bacteriophage is always present in the intestine, throughout the whole animal series, and that the most frequent virulence, indeed it seems constant, is for *B. coli*.

We know that the resistance of the bacteriophagous ultravirus to diverse agents of destruction is great. In vitro it stays alive for several years; its resistance against physical and chemical agents is intermediate between that of the vegetative forms of bacteria and the spore forms. It can be assumed then, that distributed with the dejections throughout the environment the bacteriophage will resist for a long time. Furthermore, thanks to its minute size, it can readily be carried about, especially by the seepage water. In fact, Dumas has found the bacteriophage in the tap water of the city of Paris, and in garden soil. I have found it in sea water collected some kilometers outside of the mouth of rivers. Beckerich and Hauduroy have found it in the waters of the Rhine and the Ill.

The bacteriophage is therefore extremely widely distributed throughout nature. Contamination by the bacteriophage, chiefly through the mediation of drinking water, is continual, both for man and for animals. In this regard we might recall that although there is but one bacteriophage, all strains unquestionably belonging to the same species, each strain possesses a particular virulence extending to a group of bacterial species often unrelated one to another, and that these virulences depend upon successive adaptations of the bacteriophage as determined by the succession of the organisms in which it has resided.

As a result of these continual "contaminations" each individual harbors in his intestine a great number of varieties of bacteriophage. In fact the interchange of bacteriophages between one individual and another is continuous. Each glass of water introduces new strains, each defecation throws them out into the outside world.

We will return to this extremely important fact.

VARIATIONS IN VIRULENCE OF THE BACTERIOPHAGE IN VIVO

Within healthy man, the virulences of the intestinal bacteriophage vary continually, but remain in general weak.

It is obvious that when I employ the term "intestinal bacteriophage" I do not wish to imply that there is but a single strain, since I have said that beyond all possible doubt there exist at the same time a very great number of different bacteriophage strains within the intestine of an individual. In speaking of an individual the term "his intestinal bacteriophage" includes the whole group of bacteriophagous ultraviruses which exist within him at the moment.

If one follows a healthy man, examining from time to time the activity of the intestinal bacteriophages, one will see that this activity is variable. From one time to another there will appear a sudden change in virulence, usually an increased virulence for some of the colon-typhoid-dysentery organisms. What do these sudden changes represent?

I have shown that the physical state (consistency) and the chemical state (pH chiefly) of the medium has an effect upon the virulence of the bacteriophage and principally upon its faculty of adaptation to parasitism for a given bacterium. It is evident that

all of the other intestinal conditions may likewise intervene, notably the concentration of proteolytic enzymes which destroy the bacteriophage as Wollman has shown, as well as the bacterial associations.

Tomaselli has examined the feces of horses subjected to varied types of food and has concluded that the type of food has an effect upon the virulence of the intestinal bacteriophage. This is probably referable to a change in reaction of the intestinal contents brought about by the food. But these intestinal factors, "physico-chemical conditions," although they may be important from the point of view of developing adaptation, do not explain entirely the fact of the acquisition of virulence for a given bacterium.

Man, and animals also, although it is more difficult to demonstrate it, show from time to time slight disturbances, often passing unnoticed, or only reflected by a transitory intestinal symptom, such as a stool of less than the normal consistency. Sometimes, the disturbance is slightly more pronounced; there may be one or two fluid stools, with even a sensation of discomfort or of pain in the abdomen which disappears within a few hours. In such cases, if one tests at such a time the virulence of the intestinal bacteriophage, it will *always* be found that it is very noticeably increased for the strain of *B. coli* isolated from the intestine, and, at the same time, there appears a virulence for another bacterium, usually an atoxic dysentery bacillus, a Flexner or Hiss strain, or a paratyphoid strain, most frequently a paratyphoid B.

But the healthy man or animal may be in other and special conditions with reference to a bacterial species, such as when he resides in a region ravaged by an infectious disease, that is to say, when the infectious organisms are to be found in abundance in its environment.

I have conducted numerous investigations upon this subject, and since in Europe I could not find conditions exactly suited to the work, the studies were carried out in Indo-China. Here is a summary of the observations thus made, both in Europe and in tropical countries.

Dysentery

At the beginning of the autumn of 1918 an epidemic of dysentery due to the Shiga organism appeared in the region of Paris. Living at that time in an infected district near Paris it was possible for me to examine repeatedly the stools of 9 persons, who, at the time, were neither infected nor disordered. In all, at each examination, I found a bacteriophage of average or strong virulence for the Shiga bacillus. Such a constancy in virulence for this bacterium never was encountered in the Paris district in normal times.

In July of the same year, I followed in detail a small epidemic of Shiga dysentery occurring in a house of correction containing about thirty young girls. I examined the feces of 11 of the inmates all in a state of perfect health at the time of examination, and in all there was a bacteriophage of high or maximum virulence for *B. dysenteriae* Shiga.

It is known that in the course of dysentery epidemics many persons, without being actually sick or often without being obliged to interrupt their normal work, are affected with slight transitory diarrhea. This occurred frequently in the course of the epidemic in Paris to which I have alluded. I examined 29 specimens of stools taken from such cases of simple diarrhea. In the 29 was a bacteriophage strongly active or of maximal potency for the Shiga organism.

Avian typhosis

I observed in France, during the years 1918 and 1919, an epizootic of avian typhosis.[1] I have shown that this disease, at least in

[1] Avian typhosis is a contagious disease which ravages the Gallinaceae. The causative agent is a bacillus, *B. gallinarum*, which shows very great resemblance to the typhoid bacillus infecting man. In fact, avian typhosis presents all of the characteristics of human typhoid fever. Both are intestinal diseases accompanied by a septicemia; but the mortality in the typhosis of the Gallinaceae is much higher than in human typhoid fever. It is unusual to see recovery in a chicken definitely affected. Typhosis is extremely contagious and spreads rapidly over large regions. In 1917, 1918, and 1919 it was present in disseminated foci throughout all of France. In the infected barnyards, the morbidity, and as a result the mortality since cases of recovery are rare, varied between 30 and 100 per cent. Avian typhosis shows still another resemblance to human typhoid fever, in that there are paratyphoses, just as there are paratyphoid fevers.

the epizootic which I observed, was almost always fatal.   At least 98 per cent of the chickens presenting the symptoms died, and in many barn-yards the mortality of the chickens actually reached 100 per cent.

From 81 examinations made in a region free of the disease, that is in a district not invaded, the intestinal bacteriophage, always present in the intestinal tract of the chicken, never showed any virulence whatever for *B. gallinarum*.   On the contrary, in regions infected with avian typhosis for a certain time, the intestinal bacteriophage of all of the chickens which resisted the infection possessed a virulence for the pathogenic bacillus (more than 100 examinations).

Barbone

Barbone is an epizootic disease of extreme infectiousness which spreads among the buffaloes.   I have mentioned it in an earlier chapter.   The pathogenic agent is a pasteurella.

In Indo-China I have shown that the intestinal bacteriophage of the normal buffalo did not usually have a virulence for the pasteurella causing barbone in regions which had been free of the disease for a number of years.   Whenever there was a virulence, it was weak.   Of 50 examinations made in a healthy district, only 3 animals showed in their intestine a bacteriophage possessing even a weak virulence for the pasteurella.[2]

It was possible to follow two epizootics which spread throughout a very wide territory.   At the end of each of these two epizootics, I gathered, at random, from different villages, the feces of a number of buffaloes which had certainly not been infected, for the mortality in Indo-China is 100 per cent of the animals showing symptoms, that is, every animal infected dies.[3]

[2] It should be said, as I have indicated at the beginning of this section, that one *always* finds in the buffalo, as in any normal animal whatever, a virulence of the bacteriophage for *B. coli* and for other bacteria of this group.

[3] It might be well to call attention to a distinction which does not seem to have been considered in statistical work.   For example, in this epizootic of barbone, about 30,000 buffaloes lived in the district, and about 10,000 died, a mortality for this epizootic of 33 per cent.   But none of the buffaloes remaining alive showed any symptoms, for all of the animals attacked, showing symptoms, died.   Barbone is always (at least in Indo-China) a fatal disease.   The mortality *in* barbone is then, 100 per cent.

In all of the cases in a contaminated region (33 examinations) the intestinal bacteriophage showed a virulence, for the most part high $(+++)$ or maximal $(++++)$ for the causative agent.

This virulence persists for a very long time in these animals. Five months after the disappearance of the epizootic it was still very appreciable in all of the survivors.

Plague

It is known that an epizootic, or an epidemic, of human plague results only from an epizootic of rat plague. It would appear then, that after an epidemic all of the rats which survived should be animals which have recovered (which must be rare, in view of the sensitivity of the rat) or animals which have resisted the contagion. After having shown that, in the localities in which plague had not been present for a period of several years, the intestinal bacteriophage of the rats never showed any virulence for *B. pestis*, I sought for such a virulence in the intestinal bacteriophage of rats captured in a locality where an epidemic was spreading. In all cases such a virulence was present, sometimes to a maximum degree.

All of these findings allow us to say, that in the course of an epidemic, the individuals which resist the contagion present in their intestine a bacteriophage virulent for the pathogenic bacterium which is the cause of the epidemic. Is there in this fact any cause and effect relation? Study of the disease shows us.

THE BACTERIOPHAGE IN THE DISEASED INDIVIDUAL

The first disease studied was bacillary dysentery. Daily examinations, from the beginning of the disease up to the end of convalescence were made in 22 cases of dysentery, of which 5 were fatal cases.

In the 17 cases which recovered, at the beginning of the disease and at the critical period, in no case did the intestinal bacteriophage manifest any virulence for the pathogenic bacillus of the patient himself, that is, for the organism isolated from the stool at the beginning of the infection. The virulence for *B. coli* was weak, at times entirely lacking.

Some hours prior to the development of clinical changes which indicated an improvement, the virulence for *B. coli* began to increase, then for the dysentery bacillus. This sequence was the same in all cases. According to the case the virulence for this last bacillus developed slowly or rapidly and the condition of the patient has, without exception, shown a constant relationship to the virulence of the bacteriophage. In the cases where the virulence was suddenly enhanced and reached quickly its maximum intensity $(++++)$ the number of bloody stools diminished, then ceased with the same rapidity, and the general condition of the patient improved in parallel.

In the cases where, on the contrary, the virulence increased slowly, convalescence required some time for its establishment.

In some cases the struggle was even more protracted, the virulence of the bacteriophage for the bacillus increased slowly but there was at the same time a parallel development in the bacterium of acquired resistance. There was thus both the increase in the virulence of the bacteriophage and the increase in the resistance of the bacterium, and the state of the patient, the number of stools and the general condition, was always strictly related to these two conditions, convalescence being established only when the virulence of the bacteriophage was sufficient to overcome the resistance of the bacterium.

Five cases died. In 4 of these, the bacteriophage remained avirulent, the dysentery bacillus developed without hindrance, the disease progressed without arrest, and the patient died.

The fifth case is of very particular interest. It was a woman, sixty five years old, infected with a Hiss dysentery bacillus. The strain was inagglutinable when taken from the body, and it became agglutinable only after several transfers. We know that bacteria which have acquired a resistance to the bacteriophage are inagglutinable by a specific serum, and this was the case here. In the stools of the patient there was a bacteriophage with a maximum virulence for all of the Hiss dysentery strains in the culture collection, but it exerted no action whatever upon the bacillus isolated from the feces of the patient. After several transfers this bacillus was, on the contrary, attacked, and at the same time it regained its agglutinability. There was therefore,

in the intestine of the patient a "mixed culture" of refractory bacteria and virulent bacteriophage. The disease became worse in spite of all of the methods of treatment which were applied; the number of stools increased up to about 150 per day, containing always a mixed culture, and the patient finally died of weakness.

At autopsy, *all of the organs* gave mixed cultures of both bacteriophage and bacterium. This is, I think, the first case noted of a septicemia due to *B. dysenteriae* Hiss.

The bacterium had acquired a total resistance to the bacteriophage. It had been able to develop without hindrance throughout the entire organism, and death was inevitable.

Typhoid and paratyphoid fever

Daily observation of 31 cases of typhoid and paratyphoid fever has allowed me to make conclusions absolutely analogous to those which I have described for dysentery. Three of these cases were fatal. At no time was it possible to detect the slightest virulence of the intestinal bacteriophage for *B. typhosus*. In all of the cases which recovered, the increase in the virulence of the intestinal bacteriophage for the pathogenic bacterium was preceded by an increase for *B. coli*. This always took place during the course of the second week. In mild or average cases the virulence for the pathogenic bacillus was likewise manifested before the end of the second week and disappeared a few days later, at the time when convalescence was established.

In the severe cases, the activity for *B. typhosus* did not appear in a vigorous manner until the occurrence of definite improvement.

In the cases with relapse, the reaction was complicated by the fact that a certain degree of resistance was acquired by the bacterium, and examinations showed that, in all of these cases, the beginning of final convalescence coincided with a sudden increase in the virulence of the bacteriophage, which permitted it to overcome the relative resistance of the pathogenic bacterium.

In all of the cases, the condition of the patient registered faithfully the different phases of the struggle which was taking place within the body.

These observations on typhoid fever have been confirmed by Hauduroy.

Avian typhosis

In avian typhosis recovery is rare. I have followed four cases, and the picture has been the same as in human dysentery or typhoid fever. In about 100 fatal cases it was never possible to show any virulence in the intestinal bacteriophage for *B. gallinarum*, but on the contrary, in the 4 cases which recovered, the virulence was quickly enhanced and reached its maximum $(++++)$ within a space of some hours.

BEHAVIOR OF THE BACTERIOPHAGE WITHIN THE BODY

All of these observations which are in accord, made during the course of different diseases, give us the explanation for the facts noted in healthy persons affected with slight transitory disturbances, or in those who resist contagion. The respective behaviors of the intestinal bacteriophage and of a bacterium which succeeds in invading the body may be summarized thus:

1. If the bacteriophage possesses in advance a true or latent (we will return to this in a moment) virulence for the bacterium, the latter is destroyed at its entrance into the body, and disease does not develop.

2. If the bacteriophage does not possess in advance any virulence, it may acquire it (as experiments in vitro also demonstrate). The bacteriophage adapts itself to bacteriophagy of this new bacterium, and this the more rapidly when this bacterium is deprived of resistance, and the more the bacteriophage itself is trained by heredity for the struggle. The more frequent the reinfections by a given bacterium, the more apt is the bacteriophage to acquire rapidly a degree of virulence sufficient to prevent, from the beginning, all growth. If this adaptation is immediate, the disease is aborted.

3. If the intestinal bacteriophage remains inactive, the bacterium multiplies, and disease takes place. Inactivity on the part of the bacteriophage may result, either from unfavorable intestinal conditions, or from the fact that the bacterium which has invaded has a certain degree of resistance, acquired in the body of another individual. And this last point is fundamental. The dominating factor of the process rests in the antecedents of the bacteria on the

one hand, and of the bacteriophage on the other. The bacterium may bring with it into the new organism which it invades a certain more or less pronounced resistance to the action of the bacteriophage, and the bacteriophage on its side, may present a certain degree of actual or latent virulence.

Whenever, as a result of the inactivity of the bacteriophage, the bacterium is able to grow, three possibilities may follow:

a. The bacteriophage may increase its virulence before the lesions become sufficient to lead to death; and if this takes place without causing conditions such as to permit the bacterium to acquire a refractory state, bacteriophagy results and recovery follows.

In the protracted forms it appears that there is produced simultaneously in the patient an adaptation of the bacteriophage to bacteriophagy and an increased resistance of the pathogenic bacterium. The struggle between the two beings goes on for a certain time, and the state of the patient registers faithfully the fluctuations in this struggle. The patient becomes convalescent at the moment when the virulence of the bacteriophage dominates the resistance of the bacterium. He succumbs if the bacterium acquires a refractory state.

b. The bacteriophage, as the result of unfavorable conditions, derived either from its antecedents or from the environmental conditions, fails to acquire a virulence. The bacterium develops without hindrance, disease follows, and the individual succumbs.

c. The bacteriophage increases its virulence, but at the same time the bacterium, either because it possessed an acquired resistance at its entrance into the patient, or because it acquires it after it has entered his body, becomes refractory. The bacterium grows with nothing to oppose it and the patient dies.

A secondary question, that of local conditions, plays a part. All disease is dominated, on one hand by the natural or acquired resistance of the pathogenic bacterium which invades the organism, on the other by the characteristics of *innumerable* varieties of the bacteriophage, continually ingested, which people the body.

The part of the body itself in the process of recovery is *minimal*, for clinical experience shows definitely that organic immunity, endogenous in nature, manifests itself only after the beginning of convalescence. The story of relapses is the proof of this.

Study of disease shows that the sole intervening principle in the process of recovery, is in vivo bacteriophagy. It is further evident that all of these conclusions apply only to *natural* disease. The study of experimental disease—an artificial phenomenon—is, from the point of view of the investigation of the causes of immunity, a scientific monstrosity.

THE IMMUNITY IS CONTAGIOUS

It has been emphasized that observation shows that disease does not take place when the bacteriophage possesses *in advance*, prior to the penetration into the organism of the pathogenic bacterium, a virulence for this bacterium. How can a bacteriophage possess a virulence for a bacterium which has not yet penetrated the individual who harbors the bacteriophage? Here are some observations which show the nature of this process.

At three different times in the course of experiments upon avian typhosis, I have observed the following fact. The epizootic was raging violently in a farmyard. Among the chickens present at the time of the examination, the feces of certain of them did not contain a bacteriophage virulent for *B. gallinarum*, in others the bacteriophage had a weak virulence ($+$). One chicken contracted the disease, but the virulence of the intestinal bacteriophage increased quickly to a maximum and the animal recovered. At this time, the epizootic ceased abruptly and experiment showed that in the feces of all of the chickens was to be found a bacteriophage of maximum activity ($+ + + +$).

What happened? It may be assumed thus. The convalescent chicken distributed its dejections throughout the environment, some of the chickens were "contaminated" with the extremely active bacteriophage which they contained. They likewise polluted the environment with their fecal matter and the "contamination" by a bacteriophage of maximum virulence was quickly disseminated through all of the chickens of the farmyard. When this contamination is accomplished the chickens become refractory and the epizootic stops. The virulence of the bacteriophage is thus maintained, for the pathogenic bacterium being also amply disseminated in the environment in the course of an

epidemic, the bacteriophage has had opportunity to reproduce, and thus remains virulent.

The following experiments confirm these deductions and show that this "contagion" of exogenous immunity is a fact.

Six chickens obtained from an uninfected region were employed in the experiment. I commenced by proving, through examining their feces daily for ten days, that in none of them did the intestinal bacteriophage have any virulence for *B. gallinarum*.

One of the chickens (no. 1) received per os 1 cc. of a culture of a bacteriophage of maximum virulence ($++++$) for *B. gallinarum*. After eighteen hours, I showed that this was present in the feces. This chicken then received 2 cc. of a bouillon culture of *B. gallinarum* daily per os. The bacteriophage remained virulent throughout all this time and up to nine days after the last ingestion of the bacterium.

Chicken 2 received by subcutaneous injection 2 cc. of the same bacteriophage culture, but ingested no *B. gallinarum*. The intestinal bacteriophage ceased to show a virulence for *B. gallinarum* after three days. Therefore, the ingestion of bacilli is essential to the maintenance of a manifest virulence.

Two chickens (nos. 3 and 4) were placed in daily contact with chicken 1. After forty-eight hours for one, and three days for the other, I found in their feces a bacteriophage of maximum virulence for *B. gallinarum*. As these 2 chickens had ingested neither the bacteriophage virulent for *B. gallinarum*, nor the culture of *B. gallinarum*, it is evident that they were "contaminated" with this bacteriophage by contact with chicken 1, certainly by ingestion of grain or of water soiled by the bacteriophage contained in the dejections of the latter. These two chickens (nos. 3 and 4) then ingested daily 2 cc. of *B. gallinarum*. The virulence of the bacteriophage for this bacillus maintained itself during the twenty-one days throughout which the daily ingestion took place. It could not be detected seven and ten days after the last administration of bacterial culture.

One month later, when I had shown the disappearance of the virulence of the intestinal bacteriophage for *B. gallinarum* (by virtue of the stopping of infecting doses), I caused these 4 chickens, together with 2 control chickens 5 and 6 (placed in a distant cage

to avoid a "contamination" by the bacteriophage of chickens 1, 3, or 4) to ingest 2 cc. of a culture of *B. gallinarum* prepared directly from the blood of a chicken dead of spontaneous infection.

The 2 control chickens died in two and three days after the ingestion. The other 4 (nos. 1, 2, 3, and 4) which had had one month previously in their intestine a virulent bacteriophage, resisted without showing any disturbance whatever. And it was possible to show the reappearance of virulence (++++) for *B. gallinarum*. We must conclude that this virulence had persisted in a *latent* state, and that it was enhanced, in vivo, by contact with the ingested bacteria. The bacteriophage having been able to effect this, the chickens did not contract the disease.

These experiments confirm that which simple observation had already shown, that immunity to a bacterium is assured at the time when the body contains a bacteriophage virulent for this bacterium. This immunity is exogenous and is contagious.

THE BACTERIOPHAGE IN THE COURSE OF EPIDEMICS

The presence in an organism of a bacteriophage possessing a true or latent virulence for a pathogenic bacterium assures immunity to the disease caused by this bacterium.

We can understand from this why, therefore, in the course of an epidemic, some susceptible individuals are not affected. The history of an epidemic is only the reproduction, within a community of individuals, of that which takes place within a single organism during the course of the disease.

A pathogenic bacterium is imported into a group of susceptible individuals. The intestinal bacteriophage does not possess any virulence, actual or latent, for such a bacterium. Thus the bacterium spreads from person to person and the epidemic extends. But the bacteriophage present in each individual does not remain passive. Within certain persons where the conditions of the environment are peculiarly favorable, there is an adaptation of the bacteriophage. It acquires a virulence and these individuals recover, and from them is distributed by their excretions a bacteriophage which is virulent and which "infects" the environment.

In so far as man is concerned, since the "contamination" takes place especially, most probably, by drinking water, it would be easy to provoke artificially such a "contamination" at the beginning of an epidemic by introducing cultures of the bacteriophage of maximum virulence for the bacterium, the cause of the disease, into the reservoirs, into the springs or the wells, according to the water utilized.

It is likewise probable that the "contamination" can take place through the air which transports the desiccated particles of the dejections.

Whether the contamination by the bacteriophage virulent for the agent of the epidemic may be direct, by contact, or indirect, by water or air, a patient is a centre from which radiates the disease, and a convalescent is a centre from which radiates immunity. The epidemic subsides, little by little, in proportion as the exogenous immunity due to the bacteriophage extends, and it stops when all the individuals exposed harbor within them a bacteriophage endowed with a true or latent virulence.

Immunity is contagious to the same degree as is the disease. The history of a case of an infectious disease is in the last analysis the history of the struggle between a pathogenic bacterium and a bacteriophage. The history of an epidemic is the story of this same struggle taking place in a community of susceptible individuals.

But contagious diseases are not all intestinal diseases. Among the diseases which have been studied, some—typhoid fever, typhosis—are accompanied by a septicemia. Others, such as barbone are purely septicemic conditions, and in plague, the bacterium is found localized in the buboes. How can the intestinal bacteriophage act in such diseases?

In my first communication on the bacteriophage I noted that the bacteriophage could be isolated sometimes from the urine. The bacteriophage must, therefore, be able to pass into the circulation.

Studying next an experimental disease caused in the rat by *B. typhi murium*, I saw that the bacteriophage passed into the circulation and brought about bacteriophagy in the blood. In many of the rats resisting this infection, and in all cases of recovery, I found in the blood a bacteriophage virulent for *B. typhi murium*.

In avian typhosis, in the chickens which recovered, I noticed the passage of the bacteriophage into the circulation and shortly afterward the septicemia disappeared.

Hauduroy has reported the same fact in human typhoid fever. Other authors have found the bacteriophage virulent for the staphylococcus in the pus of healing furuncles.

All of these observations show that the bacteriophage does not remain localized in the intestine, but is able to pass into the circulation and exercise bacteriophagy in any tissue whatever.

MODE OF ACTION OF THE BACTERIOPHAGE

In all that has been said we have not dealt with hypotheses. All the experiments, all the observations, admit of but a single interpretation.   The bacteriophage is an active agent of immunity. But its action is manifested not by bacteriophagy alone, although this process must certainly be primordial.   Experiment shows that it may intervene indirectly in favoring phagocytosis, in cases where, without it, phagocytic activity would not be manifested.

By precipitation with alcohol one obtains the secretory products of the bacteriophage free from living ultramicrobes.   These products, the lysins, exercise a remarkable action on bacteria. They possess the property of increasing considerably the phagocytosis of susceptible bacteria.   This action can be demonstrated in vitro.

Produce in a guinea-pig, by an intraperitoneal injection of bouillon, the formation of an exudate rich in leucocytes.   Remove this exudate, centrifugalize it, wash the sedimented leucocytes and suspend them in physiological saline.

Prepare a control mixture, composed of one part of the suspension of leucocytes, one part of the suspension of the bacterium under study, and one part of bouillon.   For the test, prepare a comparable mixture, but replace the bouillon by a culture of the bacteriophage. The tubes of both sets are incubated at 37°C. for fifteen minutes, and microscopic preparations are made.   The number of bacilli contained within the leucocytes of the smears are counted.   By comparison of the two figures obtained it is possible to appreciate the favoring action of the bacteriophage upon phagocytosis. In the case of the dysentery bacillus, experiment shows that while

in the control tube 100 leucocytes have ingested 36 bacilli, in the other, 1510 have been ingested. Thus, in the presence of the lysins the index obtained is 41.9.

In the case of the bacillus of bovine hemorrhagic septicemia, 100 leucocytes ingest no bacteria in the control tube, while in the presence of the bacteriophage they phagocytize 271.

The opsonic action is provoked, not by the ultramicrobes themselves, but by their lysin, for, by using in the place of the culture of the bacteriophage a solution of the lysins precipitated by alcohol, comparable results are obtained.

THE BACTERIOPHAGE AS A THERAPEUTIC AGENT

The bacteriophage can be cultivated in vitro. We are therefore able to procure a bacteriophage virulent for a given pathogenic bacterium and we are able to introduce into the body of a patient, at the onset of the disease, a bacteriophage endowed with a high virulence for the pathogenic bacterium.

This method of treatment is still in its very beginnings; few as yet are the authors who have directed their experiments in this direction, but despite this, the results which have already been obtained show that there is reason for hope in this direction.

Avian typhosis[4]

During the experiments upon avian typhosis I tried the curative action of the bacteriophage upon about 100 cases of natural infection in which the mortality reached as high as 98 per cent, and in some farmyards even as high as 100 per cent.[5] The mortality was reduced to 5 per cent among the chickens which, already showing symptoms of the disease, were given orally or by subcutaneous injection a dose of 0.5 cc. of a culture of bacteriophage virulent for *B. gallinarum*. No selection was made, certain of the treated chickens were in the last hours of

[4] For a detailed account of these experiments see *The Bacteriophage, Its Rôle in Immunity*, Williams & Wilkins Co., Baltimore, 1923.

[5] Obviously this is the value of the mortality among the chickens which were sick, i.e., 98 to 100 per cent of those chickens presenting symptoms died.

the disease, and it was these birds treated late which provided those making up the total mortality of 5 per cent. The bacteriophage is able to cause bacteriophagy, that is, to assure the elimination of the pathogenic bacterium, but it can not, obviously, cure lesions already formed.

Dysentery

A culture of the bacteriophage developed at the expense of *B. dysenteriae* Shiga-Kruse, presents immediately after lysis, a strong toxicity due to the dissolved bacterial substance. This toxicity diminishes gradually, the speed depending upon the strain of the bacillus. With certain strains, the toxicity is slight, or almost nil about thirty days after lysis. At the beginning of my experiments I tried to determine what action a culture of bacteriophage developed at the expense of *B. dysenteriae*, after the loss of the toxicity, would have upon the experimental disease in the rabbit caused by the injection of *B. dysenteriae*. Consider that here we have a disease which is strictly toxic.[6] An injection of 1 cc. given one to six hours after the injection of a dose of Shiga bacilli surely fatal for control animals prevented death in a majority of the cases (8 out of 11 animals survived, all 6 control animals died).

In a second experiment, the culture of bacteriophage was not injected until 16 hours after the toxic injection. Two of 6 rabbits survived, all 4 controls died.

But here is a rather unexpected observation, which had already been made under other circumstances, namely, that the intervention was never efficacious when the injection of bacteriophage culture and of toxic culture were made *simultaneously*.

We will return shortly to the significance of this *antitoxic* immunity.

After having assured myself that the injection or the ingestion of cultures of the bacteriophage developed at the expense of different bacteria was harmless, I undertook the treatment of human cases of dysentery by the administration of cultures

[6] Unpublished experiments, as are several others cited in this chapter.

of bacteriophage developed at the expense of the dysentery bacillus.

In 7 severe cases of Shiga dysentery I administered per os 2 cc. of a culture of the bacteriophage (preserved for from twenty-five days to six months, according to the cases, before use). In 6 of these cases the bloody stools stopped within twenty-four hours, in the seventh case in thirty-six hours after the administration of the bacteriophage. And from this time the patients became convalescent.

Da Costa Cruz[7] administered the same bacteriophage per os, in the dose of 2 cc. to 24 cases of bacillary dysentery. In 22 cases the stools ceased to be bloody in twenty-four hours (improvement was in the majority of cases manifest after four to six hours). In 2 cases, after a transitory improvement, blood reappeared in the stools after a few hours. A second administration of bacteriophage was followed by a definite recovery in the following twenty-four hours.

As a result of these experiences, the Instituto Oswaldo Cruz has prepared since then cultures of bacteriophage active at the same time for Shiga, Flexner and Hiss strains of *B. dysenteriae*, and has distributed them to physicians for the routine treatment of bacillary dysentery.

In the United States, McKinley has likewise obtained comparable results.

A very important, even fundamental, point which applies to dysentery, and indeed to any other disease whatever, is this— *the bacteriophage employed for curative purposes should obviously possess a maximum virulence*. That is, in a dilution of 1:1000 within a period of twelve hours as a maximum, it should produce a complete lysis of a bacterial suspension containing 250 million per cubic centimeter, and this lysis should be permanent in at least 9 out of 10 tubes. Furthermore, the lysed bacterial culture, which becomes a bacteriophage culture, ought to be filtered through a bougie, lest resistant bacteria be present, as is always possible.

Otto and Munter, and Davison, have reported that their attempts at treating dysentery by means of the bacteriophage

[7] Brazil med., 1923, **37**, 298.

were failures. These authors made their attempts at the beginning of their experiments upon bacteriophagy, without probably recognizing to what degree the virulence of the bacteriophage could vary. (Furthermore, as they had the preconceived idea that the active principle was a ferment the idea of virulence could not, therefore, be comprehended.) They certainly employed for curative purposes a bacteriophage which was but slightly active, and for this reason may have failed to obtain favorable results. Indeed, with such a bacteriophage, a strain of low virulence, favorable results can not be expected, for such a strain may even favor the development of a resistance in the pathogenic bacterium. Added support is given to the hypothesis that this is what happened by the work of da Costa Cruz, whose experiments are mentioned above. Two years ago, in his first paper on the subject, that is, at the beginning of his work upon the problem, he reported the therapeutic use of the bacteriophage in dysentery, stating that no therapeutic value attended such treatment. He also, most certainly, appreciated insufficiently the nature of the phenomenon of bacteriophagy, since he dealt only with a strain of low virulence.

Typhoid and paratyphoid fevers

In these diseases treatment is attended with a very considerable difficulty which is not present in dysentery. The dysentery organisms belong to a species which I have termed "homogeneous" with regard to the bacteriophage, i.e., a bacteriophage virulent for one, or for any strain of this bacillus, is active upon all others. With typhoid and paratyphoid strains it is quite different. A bacteriophage active for one strain is not so for all. These are "heterogeneous" species. If the bacillus of the patient is not attacked by the bacteriophage employed in treatment the effects may be entirely negative. But one encounters some strains of the bacteriophage which show a virulence much more extensive than others. The ideal would be to find a strain so polyvalent that it would attack all races of *B. typhosus*. It has not yet been found, but such strains certainly exist. The staphylococcus is also an heterogeneous species with respect to the bacteriophage, but Gratia has succeeded in isolating a virulent bacteriophage

active for almost all staphylococci. Recently I have isolated a bacteriophage which is active for about 80 per cent of *B. typhosus* strains. It is necessary, however, to state further that in so far as typhoid fever is concerned, there is still an unknown factor to be solved. Even when the bacteriophage is virulent for the pathogenic bacillus, in some cases the cure is *immediate*, in other cases a therapeutic effect is entirely lacking, and no cause can be assigned for this difference.[8] We will consider this further in Part Four of this book.

However this may be, Beckerich and Hauduroy[9] have carried out the treatment of 8 cases of typhoid fever, either by ingestion or by the subcutaneous injection of cultures of the bacteriophage developed at the expense of *B. typhosus*. In 2 cases the effect was nil. In the other 6, the effect was, it might be said, brutal. One to two hours after the administration of the bacteriophage there occurred a crisis, with sweating and defervescence, and convalescence appeared within forty-eight hours.

In 2 very severe cases of paratyphoid fever in infants the same result was obtained. A crisis, with sweating and defervescence occurred within forty-eight hours.

It might be noted that in all of the cases treated by these authors the treatment was applied during the maximum development of the disease, the blood cultures being positive at the time of treatment.

Alessandrini and Doria[10] have treated 18 cases of typhoid with comparable results. In 9 cases no effect, in 9 others a quick defervescence followed by recovery.

B. coli infections

Here, the attempts have been much more numerous, for the treatment is actually routine in certain hospitals of Paris in cases of pyelonephritis, cholecystitis, and pyelocystitis. From

[8] Compt. rend. Soc. de Biol., 1924, **90**, 25; and Rev. de path. Comparée 1923, No. 238.

[9] Rev. de path. Comparée, 1923, No. 238; and among others, Philibert, Clinique et Laboratoire, 1924, January 20.

[10] Policlinico, 1924, (sez. prat.) **31**, 109.

the observations made[11] it appears that with the express condition
that the bacteriophage administered be virulent for the colon
bacillus of the patient (this can be determined in advance) one
obtains very quickly the disappearance of symptoms and the
sterilization of the infection.

To give examples, I will cite 2 cases of my own.

A woman thirty-two years old, affected with cholecystitis, had been in
the hospital for six months. During this time her temperature had varied
between 37.8 and 39°C. The infection had resisted all of the usual modes of
treatment, including vaccine therapy. At the time of the treatment with
the bacteriophage the patient was emaciated and extremely weakened.
Two hours after the injection of 2 cc. of a culture of bacteriophage
developed at the expense of a *B. coli* strain of the *culture collection* the
temperature rose rapidly to 40.5°. The temperature remained stationary
at this level from the seventh to the eleventh hour after the injection.
Then defervescence took place rapidly, without generalized disturbance
but with profuse sweating. Twenty-four hours after the injection the
temperature was 37.2° and the patient had started upon convalescence.
She left the hospital twelve days later, apparently permanently cured.
A woman, thirty years old, had been affected with a cystitis due to *B.
coli* for about seven years. Every treatment, including autogenous vaccine
therapy had been successively tried without any result. A suspension of
250 million *B. coli*, the strain isolated from the urine of the patient, was
completely lysed in eight hours by a bacteriophage originally derived from
the intestinal tract of a cholera convalescent, but which had been main-
tained for three years at the expense of different strains of *B. coli*. The
patient received, at forty-eight-hour intervals, two injections subcutane-
ously of this culture of bacteriophage, each of 2 cc. No obvious effect was
noted. Fifteen days later, the urine was still loaded with *B. coli* as before.
At this time the patient was given lavage of the bladder with sterile
water containing 20 cc. of the same culture of the bacteriophage which had
been injected previously without success. Five days later the urine was
sterile. Recovery was complete, and six months later reëxamination
showed that the urine was bacteriologically sterile.

This last case is particularly interesting in that it shows what
importance the mode of administration may have. For if one
had been content with the injections, one would have concluded,
naturally, that they were a complete failure. But quite wrongly,
for it was the mode of administration of the bacteriophage which
was defective, not the efficacy of the bacteriophage. In so far as

[11] Among others; Hauduroy, and Philibert, loc. cit.

urinary tract infections are concerned, it is essential to combine injections with bladder lavage.

Staphylococcus infections

The first attempts at bacteriophage therapy of staphylococcus infections were those published by Bruynoghe and Maisin, but Gratia[12] has studied this treatment in a systematic manner.

In this Gratia has not employed what might be called an "auto-bacteriophage," that is to say, a bacteriophage cultivated at the expense of the staphylococcus isolated from the patient himself. As I have indicated above, this author has succeeded in isolating a bacteriophage polyvalent for the staphylococcus, that is, a strain which will cause the lysis of any strain whatever, albus or aureus. There is no need, therefore, to determine in advance if the bacteriophage which one proposes to utilize is active for the pathogenic germ of the patient. This is indeed a very great advantage, and there is reason to hope that the same will be discovered for all species of bacteria—bacteriophages which will act on any strain whatever of the given bacterium, as is the case for dysentery bacilli and *B. pestis*. The ideal, which hardly seems possible, would be to find an "omni-virulent" bacteriophage, that is, one which would cause bacteriophagy of any bacterium of any species.

In 15 cases of folliculitis, of furunculosis, of subcutaneous abscess, of anthrax, some very severe, Gratia has given three injections of 0.5 to 3 cc. at short intervals (forty-eight hours between the first and the last) of the bacteriophage developed at the expense of a stock strain of *Staphylococcus albus*. These are his conclusions:

> At the point of injection there develops an erythematous swelling, accompanied by pain and itching. This extends usually over an area the size of the palm of the hand and persists for twenty-four to thirty-six hours. A very sharp reaction of congestion is likewise to be observed at the level of the lesion, which swells, and becomes surrounded by a very marked erythematous zone, sometimes giving the passing impression the day following the injection, that the infection is rather aggravated. . . . .
>
> Following the local reaction, which persists for twenty-four to thirty-six hours, there is a very pronounced diminution in the pain and a quick

[12] Bull. l'Acad. roy. de mèd. de Belg., 1922, 5, s., ii, 72.

disappearance of the erythema, which, before it disappears, assumes a bluish tint. There is also an early softening of the induration and a resorption of the edema. Very often the centres of the abscesses, the purulent masses, undergo a rapid liquefaction and flow out through a narrow orifice, or even at times they absorb gradually little by little without leaving a scar.

Bruynoghe and Maisin, Hauduroy, Bastin,[13] and I have made similar observations.

Gratia makes mention of the fact that 6 months after the treatment but a single recurrence had taken place.

An extremely important point, to which we will revert, is that it is not necessary in any case to give more than two or three injections, and especially, that the interval between the first and the last injection should not be greater than forty-eight hours. This statement, the significance of which will appear in a subsequent paragraph, applies to all infections. Two injections may be given with an interval of forty-eight hours, or, better, three injections on *successive* days.

Treatment of wounds

McKinley began this mode of treatment[14] and he has published the results obtained in 5 cases of massive infection with the staphylococcus, of several weeks duration. Here is a summary of one of these cases, as an example, and it is interesting from several points of view.

On November 24, a man, thirty-eight years old, as a result of the fall of a plank, suffered a complicated fracture of the right forearm, with crushing of the tissues. At operation a fragment of radius, 10 cm. long was removed. On the following twelfth of February, a new operation was necessary because of the condition of the wound which had been grossly infected from the beginning, that is, for four months. The patient was taken to the hospital. The evil smelling suppuration was such that it was necessary to change the dressings, completely soaked with pus, two or three times a day.

On February 26, there having been no improvement, he received 2.5 cc. of a staphylococcus bacteriophage, injected in part into the wound and in part into the surrounding tissues.

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[13] Thesis, de med. Lille, 1923.

[14] Arch. Int. Med., 1923, **32**, 899.

February 27, a few hours after the injection, the suppuration had practically stopped. A second injection was given like that of the evening before.

February 28, the condition was already markedly improved. A third injection was given.

February 29, the condition was further improved. Granulation began. At 10:00 a.m. 4 cc. of the bacteriophage was injected deeply into the wound. At 1:00 p.m., there occurred malaise, and a chill, with a temperature of 40°C., and a pulse of 120. At 4:00 p.m. the temperature had fallen to 38.8. At 7:00 p.m. it was normal. The wound was in good condition, without suppuration.

March 4. Cicatrization was proceeding well, and the patient left the hospital.

We will return to the significance of this reaction.

Streptococcus infections

McKinley was also the first to treat streptococcus infections by means of the bacteriophage. He has treated such infections with complete success, the improvement being as rapid as in the case of the staphylococcus wounds. One case of streptococcus abscess of the lung received bacteriophage culture directly into the cavity, after drainage.

After mentioning these facts, I think that the conclusion may well be brief. Treatment by means of the bacteriophage offers a method for the specific treatment of bacterial infections. I hope soon to be able to apply this method of treatment to cholera and to plague.

I can not better end this section than by citing a very curious "prediction" of a man of a highly developed philosophical intelligence, the ingenious German, Siemens.[15] In his memoirs, written in 1892, with reference to the work of Koch upon the treatment of tuberculosis by tuberculin, Siemens wrote that he did not think that it would ever be possible to cure a disease by means of products secreted by a bacterium. He believed that when we had a general method for the treatment of specific infectious diseases that the day would have come when we had found "a parasite of these bacteria." The facts given above show that he saw correctly.

[15] I have recently become acquainted with the writings of Siemens through reading a memoir by Otto (Ergeb. f. Hyg., 1923, vi.).

THE BACTERIOPHAGE AS A PROPHYLACTIC AGENT

If it is interesting to be able to cure a disease, it is still more so to be able to prevent it.

We have seen that all of the experiments demonstrate that the individual who harbors in his intestine a bacteriophage virulent for a given bacterium, finds himself by this fact protected from the disease caused by this bacterium. Since we can cultivate in vitro a very virulent bacteriophage, it is but natural to try to determine whether the introduction into a susceptible individual of such a bacteriophage will procure for him a refractory state. Upon this point I will be brief, for nothing has been published along this line since the publication of the book[16] in which I described my experiments upon this subject.

I would simply recall that with the collaboration of Le Louët, Chief of the Veterinary Service of Cochin-China, it was possible for us by a *single* injection of 0.25 cc. of a bacteriophage for the pasteurella of barbone to confer upon a buffalo such an immunity that twenty days later the animal resisted without any disturbance the injection of 2000 fatal doses of a culture of this pasteurella. Le Louët has proved that fourteen months after such a single immunizing injection 66 per cent of the animals still resisted the injection of 500 fatal doses.[17]

Since these experiments, this mode of immunization has been applied by Le Louët on a large scale in Indo-China. And two years after the beginning of the application of the method, with thousands of buffaloes vaccinated, none have as yet contracted barbone.

In avian typhosis, in the natural disease, it appears from experiments carried out on more than 2000 chickens that the injection or the ingestion of 0.25 cc. of a *B. gallinarum* bacteriophage causes the epizoötic to stop from the time of the vaccination. A year afterwards, none of the vaccinated chickens had contracted the

[16] *The Bacteriophage, Its Rôle in Immunity*, Williams & Wilkins, Baltimore, 1923.

[17] In an earlier chapter I have shown that the contagiousness of barbone may be due to a factor still unknown. It is none the less true that the pasteurella is certainly the immediate cause of the disease. An animal immunized against the pasteurella does not contract the disease, whatever may be the reason for its contagiousness.

disease, although the epizoötic continued to rage in the district, particularly in the neighboring barnyards held as controls.

The frequency of recurrences in staphylococcus infections is well known. Gratia has stated in his paper that in 15 cases treated by the bacteriophage but a single case had showed a recurrence after six months.

Prophylactic treatment by means of the bacteriophage should be immediately applicable to many diseases caused by bacteria susceptible to the bacteriophage—typhoid fever, cholera, plague, among others, to mention only human infections.

EXOGENOUS IMMUNITY

Study of the process of recovery in natural disease, study of the behavior of susceptible persons during the course of an epidemic, the fact that the administration to a patient of a bacteriophage virulent for the pathogenic bacterium brings about recovery, that the introduction of the same bacteriophage into the body of a susceptible individual renders that person refractory, all these things show that the bacteriophage is an important agent of immunity.

The bacteriophagous ultramicrobe is foreign to the body, and it is for this reason that I have termed this immunity "exogenous," reserving the term "endogenous" for the immunity which emanates from the body itself, phagocytic and antitoxic immunity.

But in all instances where the bacteriophage is operative is the action always the same?

A culture of the bacteriophage is always a suspension of the ultravirus which has developed at the expense of the bacteria. Such a culture contains therefore both the bacteriophage and the bacterial substance dissolved and *modified* in the process of bacteriophagy. The immunizing action may therefore be due, either to the bacteriophage as such or to the modified bacterial substance or to the opsonic action exercised by the metabolic products of the bacteriophage. In other words a culture of the bacteriophage may be effective by bacteriophagy, by increasing the phagocytic action, or by provoking within the body the formation of antitoxins. Of these three actions which one predominates?

In the process of natural recovery all observations show clearly that the bacteriophage acts directly by causing bacteriophagy in

vivo. The disappearance of the pathogenic bacteria, the immediate reason for the recovery, always coincides exactly with the acquisition of virulence by the bacteriophage.

Study of recovery caused by the administration of the bacteriophage confirms the fact of the direct action of the bacteriophage in the process of recovery.

In dysentery there can be no possible doubt as to the mode of action of the bacteriophage. Introduced into the digestive tract only a single mechanism is possible: Bacteriophagy in the intestinal contents and in the mucosa. It is the same in cystitis, where bacteriophagy takes place in the bladder. I might recall in this connection the case cited in which direct introduction into the bladder was followed quickly by sterilization.

In other diseases, as cholecystitis, and typhoid fever (when effective), the effect commences to appear within two or three hours after the injection, making it very unlikely that the mode of action of the bacteriophage is any other than direct.

In the case of staphylococcus infections again, the rapidity of the effect is such that it hardly appears probable that the results can be due to the dissolved bacterial substance. As is known, if any effect is obtained with autogenous vaccine therapy it appears only after a long series of injections. It would seem from this that the action of the bacteriophage is entirely different. Moreover, in the case of infected wounds, the improvement which takes place *almost immediately* after the first introduction of the bacteriophage into the wound, shows indeed that it exerts a direct action by bacteriophagy, leading quickly to sterilization.

In processes of *recovery* the bacteriophage intervenes directly by causing bacteriophagy. To this primary action must be added the opsonic activity, for secondarily phagocytosis is stimulated.

In the case of *prophylactic* immunization the process is, on the contrary, certainly more complicated.

If a buffalo is injected with 0.25 cc. of a bacteriophage developed against the pasteurella this is what is found. The animal becomes immune *immediately* and remains so during twenty-four to thirty-six hours. Then it becomes as susceptible as is a normal animal. After about twenty days, *suddenly*, the immunity reappears and is of such a degree that the animal can resist without any

disturbance the injection of several thousand lethal doses of a culture of the pathogenic bacterium. This condition is retained for at least a year.

We have here, then, immediately after the injection of the bacteriophage a transitory immunity persisting for twenty-four to thirty-six hours, a return to sensitivity, and then twenty days later, the sudden appearance of an immunity of long duration.

The period of transitory immunity which follows immediately after the injection of the bacteriophage is certainly due to the direct action of the bacteriophage, whose presence in the blood can be demonstrated for precisely this period of twenty-four to thirty-six hours. After this time it disappears, and at the same time the animal becomes susceptible.

When, after about twenty days, the final immunity appears, the bacteriophage is absent. Thus it is not itself the cause of this permanent immunity, and experiment shows that such an immunity is of the endogenous type, that is, antitoxic. The blood of immune animals, injected into normal susceptible animals, confers upon them a strong passive immunity. Here is an experiment which shows that such blood contains an antitoxin. The rabbit is susceptible to the toxin secreted by the pasteurella of barbone. The injection of 2 cc. of a culture filtrate kills in less than forty-eight hours. I have injected 4 rabbits with 1 cc. of buffalo serum, collected twenty-five days after the animal had been injected with 0.25 cc. of a bacteriophage culture (serum preserved in a sealed ampoule for sixteen months), and, three hours later, with 2 cc. of the culture filtrate of the pasteurella. The 4 rabbits resisted, while 2 controls died in forty and forty-seven hours respectively.

It is then certain that the injection of a dose as small as 0.25 cc. of a bacteriophage culture causes in the buffalo, after an incubation period of about twenty days, a powerful and permanent antitoxic immunity.

From these experiments I have concluded that recovery can only be explained in the following manner. In the process of bacteriophagy the bacterial substances are dissolved and modified in such a way that they are then in such a physical and chemical state that the cells of the body, the producers of antitoxin, react

to them in an extremely energetic manner. Is the action of the bacteriophage limited to this? We will consider this in a moment.

From all of these experiments I have drawn the following general conclusions:

1. That in the susceptible individual, possessing no endogenous acquired immunity, the bacteriophage acts directly by bacteriophagy. The result is that the susceptible individual is rendered refractory to the disease from the moment when it harbors within its body a bacteriophage virulent for the agent of this disease.

2. That in the person affected with an infectious disease the bacteriophage acts likewise directly by bacteriophagy, and probably also indirectly by virtue of the opsonic action of its metabolic products.

3. In the establishment of endogenous immunity, acquired naturally as the result of an attack of a disease, or experimentally by the injection of a culture of the bacteriophage, the bacteriophage acts indirectly by dissolving the bacterial substance and by bringing it into the particular state which stimulates the formation of antitoxins by the cells of the body. All of the newly disclosed facts confirm these deductions.

But I have likewise concluded that, although the action of the bacteriophage is predominant in the preceding cases, in natural species immunity, that is, in the immunity which is inherent in an animal species as regards a given disease, the bacteriophage plays no rôle. There are, however, disturbing facts, as indicated in the following, which modify this last conception. The rôle of the bacteriophage is certainly most complicated and still more involved than I at first believed.

ANTIPHYLAXIS

Bordet has observed that if one repeatedly injects a rabbit with a culture of the bacteriophage, one brings about the appearance, in the fluids of the rabbits, of an antibacteriophage property. The serum derived from such a rabbit hinders the process of bacteriophagy in vitro. Bordet has concluded that this antibacteriophagic serum destroys the bacteriophage. I have shown that in reality no destruction of the bacteriophage occurs, there is simply an inhibition of its action.

An extremely curious fact, first noted by Otto, and later studied by Janzen and Wolff, is that the inhibition of bacteriophagy is only produced against the bacterium at the expense of which the bacteriophage injected into the rabbit had multiplied. Thus, Janzen and Wolff take a bacteriophage virulent at the same time for *B. typhosus* and for *B. coli*. They inject the rabbit with this bacteriophage grown with *B. typhosus*. After a few hours of contact with the serum of this animal the bacteriophage is no longer able to cause bacteriophagy in a suspension of *B. typhosus*, but on the contrary it retains its complete virulence for *B. coli*.

Aside from the apparent strangeness of this phenomenon, this experiment gives an absolute proof that the antiserum does not destroy the bacteriophage. The action of the serum does not manifest itself *on the bacteriophage itself*, but certainly upon the bacterium which is rendered refractory to the action of the bacteriophage.

This brief exposition of the properties of the serum of animals which have received *repeated* injections of a culture of the bacteriophage assists us in understanding the following afcts, apparently paradoxical.

I have observed, and mentioned, that the repeated injection of cultures of the bacteriophage, far from conferring an immunity, creates a state of hypersensitivity toward the bacterium at the expense of which the injected bacteriophage was developed.

In barbone, the larger the injection of the culture of bacteriophage the greater the delay in the immunity. If the dose injected passes beyond 25 cc. the animal acquires no immunity. And it is the same if two injections are given. In a word, immunity is produced only when a single and minimal dose is injected.

I have since returned to this question. I have shown that the blood of mice which received 5 injections, subcutaneously, at seven-day intervals, of 0.01, 0.02, 0.05, 0.01, and 0.015 cc. of an *old* culture of the bacteriophage developed at the expense of *B. dysenteriae* Shiga is endowed with antibacteriophagic properties and that these mice become at the same time hypersusceptible to the dysentery organisms *or its toxin*. A dose equal to a fifth of the dose lethal for the normal mouse kills.

A single injection of the bacteriophage immunizes, several injections at infrequent intervals sensitize.

This contra-immunity, this antiphylaxis, may be conferred passively. Mice which have received a dose of dysentery toxin equal to one-tenth of the lethal dose and at the same time 0.1 cc., also subcutaneously, of the serum of a rabbit prepared by repeated injections of a bacteriophage active for *B. dysenteriae*, die forty-eight hours later, while the control mice which have received only the toxin show no disturbance.

These mice, passively "contra-immunized" show the same hypersusceptibility to injections of dysentery bacilli as to the toxin. In this case, it is observed that they present before death a paralysis of the posterior portion of the body, indicating that death is caused by the toxin.

We have seen previously that the immunity conferred by an injection of a culture of the bacteriophage is transmitted by the serum. We now see that "contra-immunity," antiphylaxis, is likewise transmitted.

The preceding experiments force us to one conclusion. An animal on which is conferred, either actively or passively, anti-bacteriophage immunity is "contra-immunized" against the cells *or the toxin* of the bacterium with which the bacteriophage was developed.

Correlating the facts of the experiments already mentioned, which show that, in the rabbit, an injection of a dysentery bacteriophage causes a clear-cut effect upon the experimental disease *even though toxic symptoms have already appeared*, it would seem that they can be interpreted in only one way.

Within the body of an animal, a toxin must fix itself to certain cells possessing a coefficient of adsorption for this toxin or be captured by the ameboid cells. The toxin adsorbed or captured is next transformed, slowly and progressively, into antitoxin by the influence of some cellular process. In the process of bacteriophagy a similar action takes place under the influence of the products of metabolism of the bacteriophage leading slowly to the production of an antitoxin. A culture of the bacteriophage produced at the expense of a toxic bacillus must contain immediately after the lysis all of the bacterial toxin, then, gradually there occurs a transformation of this toxin into an antitoxin.[18]

[18] Perhaps an "apoantitoxin."

The injection of animals with such antitoxic products causes the formation of an anti-antitoxin, favoring the action of the toxin, and explaining the hypersusceptibility. This is, however, only an hypothesis; further study of antiphylaxis will show if it is correct.

However this may be, there are other experiments which show the generality of this phenomenon of hypersusceptibility.

Hauduroy gave a series of young rabbits 7 injections of 2 cc. of a staphylococcus bacteriophage, at five-day intervals. These animals, although all treated in the same manner, behaved in two distinctly different manners.

A certain number remained, in appearance, normal, but when injected some days after the last injection of the bacteriophage with a very small dose of staphylococcus culture (harmless for normal animals) these prepared rabbits died in less than twenty-four hours of a staphylococcus septicemia.

The other animals of the series became spontaneously emaciated and cachectic, and died. At autopsy they showed multiple subcutaneous abscesses, with no tendency to healing, in which the pus swarmed with staphylococci. As these animals had not received any injection of staphylococci they had died of a natural staphylococcus infection.

The rabbit is inherently refractory to natural staphylococcus infection. Just as soon as the fluids show the antibacteriophage property they become so hypersusceptible that the first staphylococcus that penetrates into the body grows there without hindrance.[19] Aside from all hypotheses, the fact of contra-immunity demonstrates that an animal enjoying natural immunity, may become extremely sensitive at the time when the fluids show the homologous antibacteriophagic property. When the exogenous

[19] I might remark that this single fact is sufficient to demonstrate that the process of recovery obtained by the injection of the bacteriophage, is in no way comparable to that which takes place, sometimes, as the result of the injection of killed bacteria. In furunculosis, for example, which is the single infection where vaccine therapy seems to give results, a series of injections (often 20 or more) are necessary to obtain even a beginning effect. If one does the same with the bacteriophage, i.e., gives repeated injections, one becomes sensitized to the disease! In the case of vaccine therapy, it is possible to cause (not always) a phagocytic adaptation of short duration. With injections of the bacteriophage one causes bacteriophagy in vivo.

immunity due to the bacteriophage has disappeared, endogenous immunity either natural or acquired, derived from the body itself, is unable to hinder the development of the bacterium. This is shown by the fact that, in all cases, endogenous immunity is subordinated to exogenous immunity.

CONCLUSIONS

Study of the phenomena provoked in vitro by the bacteriophagous ultramicrobe, study of the reactions of immunity in the susceptible individual, observation of epidemiological facts, and the recognition of the power of conferring immunity and of aborting the already established disease by the introduction into the body of cultures of the bacteriophage, all agree to show that the bacteriophage assures the protection of the susceptible individual exposed to contagion and the defense of this susceptible individual in the course of natural infection.

Parasitic of bacteria, the bacteriophage intervenes *directly* to destroy the pathogenic bacteria which attempt to invade the body. Secreting lysins endowed with a powerful opsonic action, it renders possible the education of the phagocyte and leads to the establishment of organic antibacterial immunity. Dissolving the bacterium and placing it in a physical and chemical state particularly fitted to react upon those cells of the body which produce the antitoxins, it initiates the establishment of organic antitoxic immunity.

Antiphylaxis shows, finally, the fundamental rôle of the bacteriophage. The appearance in the body of an antibacteriophage property causes hypersensitivity, even in naturally refractory individuals, and permits the bacterium to develop without any inhibition. Exogenous immunity being overcome, endogenous immunity, either natural or acquired, is powerless.

The bacteriophage plays a preponderant rôle in all of the phenomena of immunity. It is because of its presence that when exposed to infection an individual remains unscathed, and it is because of its presence that an individual, when sick, recovers.

Such are the facts, quite aside from theory.[20]

[20] Recognition of the characteristics and of the rôle of the bacteriophage suggests a very simple hygienic procedure which, however, might be of very great significance. During certain types of epidemics it is generally recommended that all drinking water be boiled. By so doing we unquestionably destroy the pathogenic organisms but at the same time we destroy the bacteriophage which may also be present in the water and to some extent virulent for the pathogenic bacterium. A more rational method of treatment is to subject the water to filtration through any of the efficient commercial filters (Chamberland, Berkefeld, Mandler, etc.) which retain the bacteria.

In this manner the drinking water is deprived of all pathogenic bacteria (of greatest importance are those which are resistant to the bacteriophage, since, from the point of view of contagion they are the most to be dreaded) although it contains the bacteriophages which pass through the filter.

It should be stated, however, that lacking an appropriate means for effecting filtration the water should be subjected to boiling, but only at times and in regions of epidemic disease. Under normal circumstances raw water, either filtered or unfiltered, is to be preferred.

PART FOUR

THE ULTRAVIRUSES AND IMMUNITY AGAINST THEM

CHAPTER I

The Ultraviruses

HISTORICAL

Of the various questions with which Biology is concerned at the present time, that dealing with the ultraviruses is the most obscure. Indeed, the situation is in such confusion that it is not clearly understood what is actually meant by the term "ultravirus," and in certain cases even the living nature of these beings is denied.

The fact has already been mentioned that to the majority of biologists a being which is invisible cannot be a thing endowed with life. Into their studies of the phenomena of life they bring the preconceived idea that only those things can possess life which are cellular; things which can be stained and subjected to microscopic examination. Apparently to them the limit of microscopic visibility determines the natural limits of living matter. This is a strange conception. Nevertheless, to perceive the importance of visual examination in determining the nature of things it is sufficient to observe what is taking place at the present time, particularly in regard to studies of the cell.

Histologists have built up a whole structure of science solely upon visibility, and now they are in the process of discovering that when they believed they were observing the details of the cell they were in reality studying phantoms. All of those details which have been revealed by the microscope were only those things brought about in the processes of fixation and of staining. That this assertion is not greatly exaggerated is evidenced by the statement of one of the most competent students in this field, Hardy, who states: "I believe that we have no proof that the structures recognizable within the substance of cells after their fixation can correspond to anything in the cell as long as it is alive. The majority of these things represent artifacts." It is impossible to express this idea more clearly.

As a matter of fact it is necessary to consider that aside from

rare exceptions, the direct examination of a being teaches us but very little. Consider what the bacteria, for example, show us through microscopic examination? Hardly anything. Little rods, or little spheres, and not much else is to be seen. If we would undertake to classify such organisms, it is not to the microscope that we turn, but to a study of their properties, and here there is no need to see the organism itself. In biology the nature of a being is determined by its behavior. Its form is an accessory.

The existence of beings of such size that it is impossible to see them has been predicted by philosophers for a very long time. The first, I believe, who had this conception was Mallebranche who, in 1674 wrote, in his work "*La recherche de la Vérité:*"

Although our imagination is dismayed by the thought, there are animals ever smaller and smaller, even to infinity. Our vision is limited, but it can not limit its object. . . . . The little animals of which we speak may perhaps have other smaller animals which devour them, and which to them are imperceptible because of their extreme smallness, in the same way that the first are to us imperceptible . . . . , for, indeed, the small animals do not feel the need of the microscope as much as the microscope needs the small animals.

Mallebranche, something over two centuries ago predicted the existence of pathogenic bacteria, and even the existence of the bacteriophage, although indeed, certain modern spirits have not yet reached the stage of comprehending it.

In 1881, Pasteur clearly stated with regard to the agent of rabies that if this virus could not be disclosed by the microscope it was simply because its dimensions were too small for the limits of visibility of this instrument.

In 1893 Iwanowski showed that a disease of tobacco, a mosaic, characterized by a withering of the leaves, was a contagious disease, the causative agent of which passed through filter candles. Two years later Beijerinck established the extreme minuteness of the contagious element and advanced the hypothesis that he was dealing with a "contagium fluidum vivum." As a matter of fact, experiment has shown that the ultravirus is formed of corpuscles to be found in colloidal suspension, but at the period when Beijerinck advanced his hypothesis the distinction between a true solution and a colloidal sol was unknown. To Beijerinck

belongs the credit of recognizing that there exist beings endowed with special properties and especially of separating the idea of life from the older cellular conception. By virtue of this he deserves to be considered as the first scientist to really discover the ultraviruses, for we will see in a moment that the terms "filtrable microbe" and "ultravirus" are by no means synonomous. There are ultraviruses which pass through porcelain filters with great difficulty and there are bacteria, even visible ones, which pass through with great facility.

Finally, in 1898, Loeffler and Frosch demonstrated that the agent of aphthous fever of cattle passed through the Berkefeld filter.

Since that time many filtrable viruses have been discovered. All of these filtrable viruses are most assuredly not ultraviruses, for among the filtrable substances there are included undoubtedly certain protozoa, spirochetes in particular, and some bacteria which, as we will see, possess resistant forms capable of passing through porous filters. The causative agents of the following diseases belong in this category, for it is probable that they pass through an evolutionary cycle which takes place, in part, within an insect host.[1] Such are dengue fever, transmitted by a Culex, yellow fever, transmitted by a Stegomyia, tsutsugamushi disease, transmitted by a Trombidium, pappataci fever, transmitted by Phlebotomus, spotted fever, transmitted by a tick, typhus exanthematicus, transmitted by a louse, hearth fever of cattle, and sautante of sheep, transmitted by ticks.

On the other hand, certain diseases, although transmitted by insects may be caused by ultraviruses, the insect here playing simply the rôle of inoculator without taking any part in the evolutionary cycle of the parasite. Such are, pernicious anemia of horses, transmitted by a Stomoxys, horse sickness, also transmitted by Stomoxys, possibly miliary fever of man, which may be transmitted by an insect living on field mice (Chantemesse and Marchoux). There are also insects usually members of the Aphidi-

[1] Unless this evolution be "fictitious" the ultraviruses are energetically adsorbed by the red cells. It is necessary perhaps for the blood to be completely digested in order to permit the ultravirus to become free and to enable it to pass, either into the glands, or into the excreta of the insect.

dae which transmit the agents of the plant diseases termed mosaics. In the case of the mosaics the agent is most certainly an ultravirus.

Finally, many human and animal diseases are caused by filtrable microbes and are transmitted directly. The list given below although a long one certainly must be far from complete. In the list those diseases due definitely to ultraviruses in the strict sense of the word are italicized. For the rest we only know that the causal agent passes through porcelain filters, a fact which, as we will see, is not sufficient to characterize an ultravirus. All that can actually be said in regard to these last, is that the agent is a filtrable microbe, with in most instances a very great probability that they are ultraviruses, as is indicated by the other properties of the organisms.

Diseases affecting the bacteria: the *bacteriophage* (d'Herelle, 1917)

Diseases affecting plants, belonging for the most part to the families Solanaceae, Graminaceae, Cruciferae, and Papilionaceae: *mosaics* (Iwanowski, 1893)

Diseases affecting fish: variola of the carp

Diseases affecting birds: *avian plague* (filtrability shown by Centanni and Savonuzzi (1902); ultrafiltrability demonstrated by Andriewski, 1913)

   molluscum (Marx and Sticker, 1902)

   plague of blackbirds (Maggiora and Valenti, 1903)

   sarcoma (Peyton Rous, 1911)

   osteochondroma (Rous and Murphy, 1913)

   leucemia (Ellerman and Bang, 1908)[2]

Diseases affecting mammals:

   aphthous fever of cattle (Loeffler and Frosch, 1898)

   sheep-pox of sheep (Borrel, 1902)

   cattle plague (Nicolle and Adil-Bey, 1902)

   rabies, to which all mammals appear susceptible (the assumed cause an invisible microbe, Pasteur (1881); filtrability proved by Remlinger and Riffat-Bey (1903); ultrafiltrability by Levaditi. 1923)

   swine plague (de Schweinitz and Dorset, 1903)

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[2] Let us mention that these diseases have been for the most part observed among domestic birds, the chicken in particular, more rarely in the pigeon, the duck and the goose. This is undoubtedly due to the fact that these birds are more readily observed than are wild birds. This remark applies equally to the case of mammals. It would be strange if wild animals were exempt from ultravirus diseases.

cow-pox (filtrability, Negri, 1905; ultrafiltrability, Casagrandi, 1908)
catarrhal fever of sheep (Spreull, 1905)
canine-plague (Carré, 1905)
specific stomatitis of cattle (Ostertag and Bugge, 1906)
plague of guinea pigs (Petri and O'Brien, 1910. In 1913 Gaspari
    and Sangiorgi observed another disease of guinea-pigs due to a
    filtrable virus which may, perhaps, be identical with that of Petri)
poliomyelitis of guinea-pigs (Römer, 1911)
myxoma of the rabbit (Moses, 1911)
rat disease (Novy, 1911)
Naïrobi disease of sheep (Montgomery, 1911)
equine typhoid fever (Bemelmans, 1913)
bilious fever of cattle (Sergent and Lhéritier, 1919)
pustular stomatitis of sheep (Aynaud, 1922)
encephalitis of cattle (Donatien and Bosselet, 1922)
acute adrenal infection of guinea-pigs (Jonesco and Mihaiesti, 1922)
Diseases affecting man:
molluscum contagiosum (Juliusberg, 1905)
verrucae (Ciuffo, 1907)
variola (Casagrandi, 1908)
trachoma (Bertarelli, 1908)
mumps (Granata, 1908)
poliomyelitis (Landsteiner and Levaditi; Flexner and Lewis, 1909)
scarlatina (Bernardt, 1911)
measles (Anderson and Goldberger, 1911)
alastrim (Beaurepaire-Aragao, 1911)
blenorrhea of the new-born (Botteri, 1912)
coryza (Kruse, 1914)
influenza (Nicolle and Lebailly, 1918)
encephalitis lethargica (Strauss, Hirshfeld and Loewe, 1919; ultra-
    filtrability, Levaditi, 1923)
herpes (Strauss, Hirshfeld and Loewe, 1919)
nodular periarteritis (Harris and Friedrics, 1922)
varicella (Netter, 1923)

THE IMPORTANCE OF THE RÔLE OF THE ULTRAVIRUSES

Is the list of diseases caused by ultraviruses completed? Certainly not, for as we have seen, not a year passes without the discovery of the rôle played by a filtrable virus in some disease up to that time unclassified. But this is not all. The list will doubtless be augmented by unforseen additions. Although as yet very brief, the history of bacteriology already shows us the surprises which it holds in reserve.

Swine plague, or hog-cholera, has been the subject of a great deal of investigation, and all authors were at one time in agreement in incriminating a bacterium, *B. suipestifer* discovered by Salmon, as being the agent of the disease. Indeed, how could bacteriologists imbued with the ideas of Koch embodied in the "experimental proofs" have the least doubt of it? *B. suipestifer* is found in the blood, in the liver, the spleen, and in general in all organs of the sick animals. Selander had shown that the bacillus produces a toxin in culture media, and this was confirmed by many authors. Cornil and Chantemesse had succeeded in attenuating *B. suipestifer* and these attenuated cultures conferred immunity when they were employed as a "vaccine." This was likewise confirmed. Citron immunized with culture extracts, and de Schweinitz had obtained a serum presenting, according to him and to others who confirmed his experiments, a preventive power when given to hogs. This serum even had a curative action in naturally infected animals. Finally, as an incontrovertible argument, the inoculation of cultures of this *B. suipestifer* reproduced the disease, with the symptoms and the lesions of the natural disease.

What more in the way of proof could be asked for? The famous trinity of experimentation was accomplished, and with some to spare. And there are many human and animal diseases in which the rôle of the agent incriminated is far from being as solidly established. If, at that time, a scientist had announced that possibly all of this signified but little for there existed a fundamental difference between the disease artificially provoked by the injection of *B. suipestifer* and the natural disease, *essentially in that the first was not contagious, while the natural disease is contagious to a very high degree*, the reply would have certainly been that if it is necessary to stop for details of this kind bacteriological investigation would become impossible, for such a proof is not given for any of the agents held responsible for epidemic diseases, with the exception of plague. Nevertheless, this proof *alone* has a value as to the pathogenic rôle, as we will see.

The work of de Schweinitz and Dorset can hardly be too greatly admired, and to my mind, from the point of view of its importance should be placed immediately after that of Pasteur and of Koch. It has demolished the beautiful edifice of the etiology of hog-

cholera. They have shown that *B. suipestifer* is only an "associated" bacterium, according to the happy expression of Nicolle. The true pathogenic agent is a filtrable virus. *B. suipestifer*, a bacterium which is found widely distributed in the environment, multiplies in swine only through the favor of the infection caused by the filtrable virus. This fact is today universally accepted, but this agreement has not been reached without many controversies.

I have said that the single proof of pathogenism consists in the reproduction of an experimental disease presenting the same contagious character as the natural disease. This desideratum is realized with the filtrable virus discovered by de Schweinitz and Dorset. A pig infected with the filtered product derived from a pig affected with the natural disease, contracts a disease which is transmitted naturally to healthy swine which dwell with it. With this, the final proof of the rôle of the filtrable virus is established.

But, the proofs which were furnished in favor of the pathogenic rôle of *B. suipestifer* were much more conclusive than those which we have concerning the rôle attributed to certain bacteria, for example, *B. typhosus* and *Vibrio cholerae*.

A history comparable to that of hog-cholera has taken place with regard to influenza. For a long time *B. influenzae* of Pfeiffer has been accused, and indeed, many authors—although the number is continually diminishing—still consider it as the pathogenic agent. In reality the pathogenic rôle belongs to a filtrable virus which was isolated for the first time by Ch. Nicolle, and by several other bacteriologists since.

Must we consider the rôle of the "associated" organisms as of no significance? Very far from that. Sometimes the rôle is an accessory one, as in hog-cholera, where the disease caused by the filtrable virus alone is almost always fatal. In influenza, the rôle of the associated organisms—for there are several—*B. influenzae*, the pneumococcus, and the streptococcus, individually or conjointly—is of much greater importance.

In the course of the first wave of influenza in the epidemic of 1918 the disease was generally caused by the filtrable virus alone. It consisted then of a simple febrile attack of sudden onset, with

headache, persisting for from three to six hours, followed by a rapid recovery. On the contrary, in the second wave of the epidemic occurring in September, the benign influenza was very frequently accompanied by very severe secondary diseases caused by one of the associated organisms which were able to invade the body thanks to the initial infection, *which alone was contagious*. Aside from rare exceptions, the individuals who had undergone a mild attack of the contagious disease, caused by the filtrable virus only during the first wave were protected from the severe infection with complications of the second wave. They were immunized against the *fundamental* contagious disease.[3]

To explain the point a little further; in the course of an epidemic of influenza an individual affected with bronchopneumonia arrives in a city, as yet uninfected. He becomes a centre of contagion and the disease spreads with an extreme rapidity. In a non-epidemic period, a patient with bronchopneumonia, aside from rare exceptions, does not spread the disease, or at least, never becomes the centre of a generally spreading contagion. In the first case, that which the diseased person distributes is not the agent of the bronchopneumonia, the pneumococcus, the strepto-coccus, or the bacillus of Pfeiffer, it is the filtrable virus. Among the individuals which contract the simple febrile contagious disease a certain number likewise contract bronchopneumonia,

[3] Many examples of this natural immunization have been cited. Here is, I believe, one of the most typical, as brought to my attention by my colleague Wollman, who, when mobilized, was battalion medical officer in a regiment of Senegalese on the Salonika front. In May, influenza, the purely febrile form without complications, spread through his battalion, at that time retired to the rear and isolated from the remainder of the army. Everyone in the battalion contracted the disease but there were no fatal cases. In October and November of the same year, during the second wave of the disease, Wollman's battalion was at the front. The disease spread rapidly and with a high mortality throughout the battalions at his right and left (which had remained uninfected during the first wave) but there was not a single case in his battalion.

In the next pandemic (they follow one another at intervals of fifteen to thirty years, probably when the greater part of the population have become susceptible) there might be certain advantages in favoring the infection of individuals at the beginning, when the disease occurs in the febrile form only.

but here the bronchopneumonia is not caused by the imported pneumococci, streptococci, or influenza bacilli, but by those which previously existed in the environment, those which each of the individuals affected normally harbored in their upper respiratory tracts before the arrival of the patient with the contagion.

Outside of epidemics of influenza isolated cases of bronchopneumonia occur, just as isolated cases of hog-cholera develop in the absence of an epizoötic, but then the filtrable virus is not present. In certain individuals in whom the natural resistance is diminished through circumstances of any kind—circumstances which are precisely those considered as representing the "conditions of infection"—the streptococci, pneumococci, and *B. influenzae* in the first case, *B. suipestifer* in the second case, banal parasites of the body, are able to invade the impaired organism. But under these circumstances they are no longer contagious in the strict sense of the word. If such an individual is transported into an uninfected region, he will not become the focus of an epidemic.

The "conditions of infection" play a rôle comparable to that of the filtrable virus, but these conditions are personal. Not being of themselves contagious the disease does not develop.

It is probable that the great majority of the epidemic diseases, perhaps all, are of the same nature as those mentioned. They may all be mixed diseases, brought about, in part by an ultravirus, the contagious agent which by itself causes a condition more or less severe. This initial disease is accompanied always, or occasionally, according as the bacterium of the secondary infection is more or less widely distributed or more or less virulent, by an additional disease superimposed upon the first. According to the association, it may be the initial disease which is the most severe, or it may be the secondary non-contagious disease which is most destructive and which may "mask" the original infection. In a non-epidemic period, the secondary disease may exist only in certain individuals in whom is to be found a particular receptivity. But then the disease does not present the "epidemic" character; it is not contagious in the true sense of the word.

As I have already mentioned, this explanation is somewhat

embarrassed by a lack of suitable terms.   Influenza and tuber-
culosis are both contagious diseases, nevertheless, a considerable
difference can be detected between the two contagions.   The first
is really contagious, the second is simply transmissible.

I have said above that in all really contagious diseases, the
contagious agent may be an ultravirus.   The following indicates
the basis for this statement.

We recognize many epizoötic diseases, of which the agent is,
so to speak, known.   But, it is curious to observe that in the case
where the agent incriminated is an ultravirus, the experimental
disease is, without exception, contagious itself, while in those which
are attributed to a bacterium there is no tendency to dissemina-
tion, although the natural disease is contagious to a high degree.
It is likewise curious to observe that this fact remains quite unex-
ploited.   Most authors appear to find this situation very natural.

We have discussed the conditions of infection in the disease of
mice due to *B. typhi murium*, mentioning that the experimental
disease is not contagious while the natural disease is an epizoötic.
What, in nature, is the contagious element in this disease?   Cer-
tainly a filtrable virus, without doubt that discovered by Novy.
Let us note that *B. typhi murium* and *B. suipestifer* are related
organisms.

Here is another fact.   The contagious character of chicken
cholera is well known.   Pasteur showed that the agent of the
disease is a pasteurella, as is assuredly true, just as a pasteurella
is the cause of barbone in the buffalo, but it is equally true that the
experimental disease is not contagious.

In so far as barbone is concerned, the disease presents itself in
two forms.   Sporadic cases occur, without any tendency toward
dissemination, and at times epizoötics develop which spread
within a few days throughout an enormous territory.   Such an
epizoötic raged in Java about thirty years ago, and within the
space of a few months, almost completely annihilated the buffaloes
of this island.   But, I have shown in the experiments described in
an earlier chapter that the pasteurella can not be the agent of the
contagion.

The conclusion must be that barbone, like chicken cholera,
considered as diseases, are certainly caused by pasteurella organ-

isms, but the contagion can only be referred to another agent which can only be an ultravirus. This appears the more probable, since pasteurella forms are the "associated" microörganisms in other animal diseases undoubtedly caused by ultraviruses, in canine plague, for example.

The situation in barbone is comparable to that in influenza. In both cases the ultravirus causes a mild disease which favors infection by an associated organism, which in turn leads to a severe disease.

As I have already remarked, one might think at first that the fact that the anti-pasteurella immunity protects against the disease shows that this bacterium is the real agent. Such a conclusion is false, for in such mixed diseases the immunity must be double. The immunity acquired naturally or experimentally against the ultravirus protects all sensitive individuals against the epidemic, but it does not protect the isolated individual against the secondary disease. That is to say, the disease may no longer be able to spread in an epidemic form although sporadic cases may still occur, just as bronchopneumonia may develop in certain individuals outside of influenza epidemics. On the contrary, the immunity against the bacterium, the associated organism, protects against the secondary disease in all cases, and indeed, if the initial disease due to the ultravirus is benign it is evident that immunity against the associated microbe is preferable to an immunity against the ultravirus. The opposite would be preferable if the disease due to the ultravirus is severe.

Moreover, we know of many diseases unquestionably caused by ultraviruses where the associated microörganisms play a certain rôle, as in scarlatina, where one can almost always isolate a streptococcus from the blood, in variola, where often a staphylococcus, at times a streptococcus, can be isolated, and in diverse animal diseases where pasteurella organisms can be recovered. Further, it is known what precautions it is necessary to take in laboratories where rabies virus is prepared, in order to avoid agonal infection of the cord of the rabbits which die of rabies. It seems, therefore, to be a general fact that diseases caused by the filtrable viruses favor infection by bacteria, those organisms which are normally parasitic upon the mucous membranes.

The extreme complexity of certain diseases is evident, where all of four factors, each important, may come into play—the body itself, the pathogenic ultravirus, an associated bacterium, and the bacteriophagous ultravirus parasitic of the bacterium.

There is then great possibility that the list of the pathogenic ultraviruses may undergo successive additions. It is on the other hand evident that if the proof of the rôle of an ultravirus is relatively easy to provide in so far as animal diseases is concerned, since it is permissible to experiment upon animals which are themselves susceptible and to place them in conditions which approach as nearly as possible the natural conditions, such proof is much more difficult in the case of human diseases, which, like cholera, and typhoid fever, are specific for man, and where, although the bacteria accused may simply be secondary invaders, they cause a severe disease which masks the initial contagious disease. Experiment upon the higher apes would allow us to solve this question, but unfortunately such experiments would be very expensive, and although people will provide generously the millions necessary to organize the collective butcheries which are called wars, scientists are unable to obtain the infinitely smaller sums needful to organize investigations such as might preserve human lives or augment well-being through the control of epizoötics.

However this may be, the single valid proof touching the rôle of a bacterium in this or that disease consists in reproducing experimentally the disease presenting the symptoms, the lesions and the contagious character of the natural disease. When this proof is not provided, the rôle of the supposed agent remains doubtful, and this is the case in all of the truly epidemic diseases attributed to bacteria, plague excepted.

Is this equivalent to saying that we must in a general way question the rôle of every incriminated pathogenic bacterium? Evidently not. For we possess quasi-absolute proofs with reference to the rôle played by a certain number of them, for example, the anthrax bacillus, the bacillus of human plague, the tubercle bacillus, the tetanus bacillus, and the diphtheria organism. For these, and doubtless for others also, their significance can hardly be questioned. Where there is a doubt with regard to the *in-*

dependent rôle of a visible bacterium, and indeed, almost the certainty of error, is in those cases where the inoculation of the suspected bacterium produces, within the naturally susceptible animal, an experimental disease of which the contagious character is different from that which appears in the natural disease. In these cases an ultravirus must intervene.

It might be added that all of the diseases experimentally induced by an ultravirus are just as contagious as is the natural disease itself. This fact is adequate to prove that the ultravirus is the specific agent.[4]

THE FUNDAMENTAL METHODS OF PROTOBIOLOGY

Detection of ultraviruses

The fundamental methods of bacteriology consist in the isolation in pure culture of the bacteria. Because of the special characteristics of the ultraviruses the technical procedures of bacteriology are not applicable to them, nevertheless experimental work can only be effected if we isolate them, and then cultivate them in the pure state. Upon what principles must we base the general methods designed to realize these desiderata?

Up to the present time in order to detect the presence of an ultravirus in tissue derived from a diseased animal the method has been to emulsify this tissue in saline and filter it through a porous filter, such as the Berkefeld or Chamberland candles. Such a procedure must fail to give a result and should be discarded, for this reason. In the first place a germ may pass through such a filter even though it is not an ultravirus, as is the case for many vibrios found in water, for the Asterococcus of bovine pleuropneumonia, and for the spirochete of infectious jaundice. In short, the porous filter allows the passage of very minute bacteria and of the filtrable forms of certain organisms which can

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[4] An important deduction can be drawn from this. The systematic destruction of harmful animals, the rat in particular, can only be effected if one employs an ultravirus such as is the natural agent of the disease (that studied by Novy, for example, in the case of the rat). The method would be, moreover, of extreme simplicity, for it would only be necessary to liberate experimentally infected animals in areas frequented by the animals. The virus could be preserved by laboratory passages.

hardly be considered as ultraviruses. And on the other hand—a situation somewhat more serious—some of the true ultraviruses are unable to pass though such porous filters, even though their dimensions are infinitely less than the pores of the filter. The reason for this is very simple. As we will see, the ultraviruses whose dimensions have been determined up to the present time have an average diameter of 20 millimicrons. This corpuscle is obviously insoluble in the liquid in which it is suspended. And from this fact it appears that an ultravirus is formed of a colloidal micella and therefore must possess the properties of micellae, chiefly that of being the bearer of a double electric charge. That is, moreover, evident a priori, for all living beings are of colloidal nature. To mention only the bacteria, microscopically visible, all of them behave like large micellae, of which they possess the characteristic essentials. That is, they possess an electric charge, whose sign has been determined for many species (usually negative), and they are adsorbed by certain substances, such as animal charcoal. These are only two of the properties. It is by virtue of the phenomenon of adsorption that the bacteria are retained by the porous filters, for it has often been demonstrated that the diameter of the pores is far greater than the diameters of the bacterial cells. It has also been shown that the pH of a bacterial suspension exerts a profound effect upon filtration. If the bacteria were not adsorbed by the walls of the pores they would pass through the filter just as a locomotive passes through a tunnel.

Let us state in this connection, that the colloidal state of the bacteria has as yet hardly come into experimental consideration. It is only with a certain sense of embarrassment that one meets with the views of certain bacteriologists who, considering the ultraviruses, appear to feel that the colloidal nature is incompatible with life. Nevertheless, it can confidently be affirmed that biology will make real progress only when the time is reached when the science is considered truly as "the science of living colloids." The introduction to the study of microbiology should not be medicine, it should be physical chemistry. But many years must pass before this will be understood. One may, however, recall that the founder of microbiology was Pasteur, a chemist.

But let us return to the question of the ultraviruses. As I stated earlier in this text it is not quite exact to say that a substance is a colloid, for the truth of the matter is that the substance is matter found in a colloidal state, because the colloidal state is not derived from a chemical property inherent in a substance, but represents a state determined solely by the two physical properties, insolubility and size of particles. The colloidal state begins to manifest itself with any insoluble substance in an ionizable medium when its diameter is below a given dimension (from 0.1μ to 5 to 6μ according to the density of the particular substance), and this quite without regard to the chemical nature of the particle. The colloidal state is increased in an inverse ratio to the masses of the particles.

Bacteria visible under the microscope show very clearly colloidal properties, but these characters are even more marked with the ultraviruses, solely because the dimensions of the latter are much smaller than those of the bacteria. The natural result is that the ultraviruses are adsorbed in an intense manner by all substances which possess the general property of adsorbing colloidal particles, substances such as animal charcoal, the infusorial earth of the Berkfeld filter and the porcelain of the Chamberland candle, among other things.

From this it is clear why we have for such a long time argued the question as to whether the germs, the agents of rabies and of vaccinia, were or were not filtrable. The reason, it appears, was simply because their passage through a porous filter was very inconstant. Sometimes they passed through, more often they were retained. In reality we will see that their dimensions are infinitely smaller than those of the pores of the tightest filter candles.

The conclusion is that all experiments concerning the presence of an ultravirus in the different diseases should be interpreted in favor of the case in question, for it is certain that, in many cases, its absence has been concluded upon the basis of the fact that the filtrates remained sterile, when, as a matter of fact, there had been an adsorption by the bougie.[5]

[5] In avian typhosis the experimental disease is not contagious, although the natural disease is so to a high degree. Since the epizootic of 1918 I have

Porous filters are excellent instruments when one wishes to sterilize a liquid (in the true sense of the word sterilize), that is to free a fluid of bacteria such as are cultivable in vitro (let us again emphasize the true meaning of the expression "cultivable in vitro," as will become plain in a moment), but they must, however, be absolutely eliminated from all experiments in which the investigator is attempting to disclose an ultravirus.[6]

How then, can we proceed? It is necessary to effect the filtration through a porous membrane which possesses to a minimum degree the power of adsorbing colloidal micellae. Such membranes are known and are routinely employed by physical chemists, who have for a long time abandoned the use of porous bougies, having shown that the inanimate colloids were held back by adsorption. The most practical are made of a membrane of collodion, and can readily be prepared in any laboratory.[7] The pores of such filters are much more minute than are those of the candles, but despite this the ultraviruses pass through readily and constantly. In this way experiments are easily conducted and are free of the element of chance.

Beijerinck had shown that a filtrable microbe, that of the mosaic of tobacco, is of extremely small size (he thought that it was present in soluble form), but it was, I believe, Casagrandi who, in 1908, showed that a filtrable virus, the agent of vaccinia, passed through collodion membranes. Andriewski next showed that the cause of avian plague was ultrafiltrable and he

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attempted to prove if the contagion was due to an ultravirus. I have concluded in the negative, since I always failed to infect chickens with the blood of sick chickens, filtered, after dilution, through a bougie. I have since recognized that this method is but a poor one, and have therefore begun again to repeat the experiments in accord with the methods presented here.

[6] The bacteriophagous ultravirus appears to be less adsorbable by the material of the filters than are the other ultraviruses. This allows the use of such filters with the bacteriophage, although ultrafilters are to be preferred.

[7] For the preparation of ultrafilters consult the texts on colloidal chemistry, particularly that of J. Duclaux, "Colloids;" Gauthier Villars, Paris, 1923.

even determined that its diameter must be about 20 milli-microns.[8]

The next step was the demonstration that the bacteriophagous ultravirus also passed through ultrafilters which allowed the passage of the micellae of serum albumin and that it was held back by membranes which retained the albumin. Prausnitz, by a method similar to that of Andriewski, has shown that this ultravirus has a diameter approximately equal to 20 millimicrons. Recently Levaditi has shown that the ultraviruses, the causative agents of rabies and of vaccinia, as well as that of encephalitis lethargica, have the same dimensions as that of the bacteriophage, all passing through the same collodion membranes. The result is that all of the ultraviruses which we have up to now measured are of about the same size.

The question of the isolation of the ultraviruses, is therefore by necessity resolved in a practical manner. The use of ultra-filtration in experiments on the ultraviruses will doubtless over-throw the conclusions of many investigators carried out with filter candles.

Cultivation of ultraviruses

The ultraviruses appear to be obligatory parasites, that is, they do not appear to be capable of reproducing outside of the body of the host which they parasitize. From this it might be assumed that culture in vitro would be impossible, and to some extent it is true.

We know that in so far as the bacteriophage is concerned it is easy to obtain a culture in vitro. Is this equivalent to saying that it is itself directly cultivable in an artificial culture medium? Not at all, for it is the bacterium, the being parasitized which is cultivable in vitro. The bacteriophage parasitic in these bacteria reproduces at their expense, and finally, when all of the bacteria present have been parasitized and dissolved, the medium, contains no living organisms other than the bacteriophage. Thus,

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[8] The unit of measurement for bacteria is, as we have said, the micron (μ), equal to one one-thousandth of a millimeter. For the ultraviruses the unit is the millimicron ($\mu\mu$), that is, a thousandth of a micron, or a millionth of a millimeter.

in this way the medium has become a true culture of the ultra-virus, although the virus itself was unable to multiply in such an artificial medium.

Is it not possible to adopt a similar technic for the study of all ultraviruses? Everything indicates that the ultraviruses are intracellular parasites; probably they can assimilate nothing but living protoplasm, and beyond doubt this is the reason why they can not be cultivated in artificial culture media. But the culture of cells has become, since the work of Harrison, Burrows, and Carrel, as surely accomplished as is the culture of bacteria. Consequently, why not seed the ultravirus which it is desired to cultivate in a culture of an appropriate tissue? The ultravirus would then be able to reproduce within the cells of its habitual host and the final result would be, as in the case of the bacteriophage, a culture in vitro of the ultravirus involved. A few experiments have, indeed, shown that such a method can be applied.

The method was first employed, I think, by Steinhardt-Harde in 1913. She cultivated fragments of rabbit cornea in the plasma of this animal and inoculated them with vaccinia virus, free of bacteria. In this way she obtained three successive cultures of the ultravirus, and the virulence of the virus maintained itself for more than thirty-four days at a temperature of 37°C., when the controls were avirulent in less then seven days. She noted that the tissue must be living. With a tissue which had undergone gelation no culture was obtained. Spleen, liver, and muscle tissues failed to maintain the culture, although with rabbit testicular tissue she was successful.

Levaditi has employed the same procedure for the cultivation of the ultravirus of poliomyelitis (1913), of rabies (1914), but by growing them in fragments of the horn of Ammon from the brains of infected monkeys. Growth did not take place when the ultraviruses were seeded in cultures of normal tissues. He noted that these ultraviruses reproduced at the expense of the connective tissue cells, and that they were cultivable only in the tissue employed. He next cultivated the ultravirus of encephalitis lethargica (1921), but in this case by inoculating a filtrate containing the pure virus into a culture of the testicular tissue of the normal rabbit.

In 1908, Marchoux apparently obtained (he was the first I think) the cultivation of an ultravirus, that of avian plague, in a medium composed of defibrinated chicken blood. Landsteiner confirmed this finding and showed further that if the red cells are killed by gel formation the cultivation can no longer be accomplished. Marchoux had, after all, accomplished the culture of an ultravirus in the presence of living cells, but as the red cell survives for some time outside of the body he had not accomplished the prerequisite condition of the cultivation of the susceptible cells.

The cultivation of ultraviruses is therefore possible.

Isolation of ultraviruses

There remains the question of the isolation of the ultraviruses, that is, the method of procuring "ultrapure" cultures by the inoculation of a single germ. Here there is no especial difficulty. It is only necessary to employ the dilution method, that is to say, to inoculate into an appropriate tissue culture a quantity of the liquid, containing the ultravirus in suspension, sufficiently small so that it will contain but a single germ. This method has been applied for the isolation of a single bacteriophagous ultramicrobe, and it is applicable in a general manner.

These general methods differ distinctly from those of bacteriology. And the science of the ultraviruses, which might perhaps be termed protobiology, should not be confused with bacteriology, the science of the bacteria. It forms with the latter, together with protozoology or the science of the protozoa, and with mycology, the science of the fungi, one of the four branches of microbiology.

That protobiology may be a science with a very delicate technic, that it may be necessary to develop a particular procedure for each individual ultravirus, is certain, but these difficulties are by no means insurmountable. It may be said with certainty that protobiology will not be within the range of everybody who, with more or less reason "works" at bacteriology. And this may not be too great an evil. Study of the ultraviruses will surely become a specialized science and those who attack it will be specialists. Clearly it is not the material for study that is

lacking. It may possibly be permissible to think that if some one some day comes to know the nature of life, it is protobiology, the science of the most elementary forms of living matter, which will reveal it to him.

PROPERTIES OF THE ULTRAVIRUSES

The fundamental property of the ultraviruses, the property which determines their general behavior, is the colloidal state in which they exist. This is due solely to their dimensions, since it is only a matter of size (joined with insolubility) which confers upon a particle this particular state of matter.

In so far as their order of size is concerned, we have seen that the question has been actually determined in the case of the bacteriophagous ultravirus, of avian plague, of vaccinia, and of the virus of encephalitis lethargica. All have the same dimensions—about 20 millimicrons in diameter.

Let us mention, however, that the smallest known bacteria are about 200 millimicrons. The diameter of the ultravirus is then, only about one-tenth of that of a bacterium. There is no limit cutting into the scale of beings, limiting them; it is an uninterrupted chain. That which characterizes an ultravirus, is not so much its magnitude, as its particular behavior.

The fact that an ultravirus has a diameter of 20 millimicrons determines a very important characteristic of its nature. It can only be constituted of a simple protoplasmic micella, permitting thus the definition of the ultravirus, sensu stricto, as a living micella. All ultraviruses function as antigens and are of albuminoid nature. They are formed of protoplasmic micellae. The ultraviruses possess a demonstrable mass, for they accumulate in the sediment during centrifugation (avian plague, -Russ; bacteriophage, -d'Herelle).

As we have already observed, the property of adsorption possessed by different substances for ultraviruses is related to the colloidal state of the latter. In this respect the following observation, made by Vallée and Carré, is interestng. These authors have rightly seen with what prudence it is necessary to interpret observations tending to show that such and such an ultravirus parasitizes the elements with which it is found associated. Thus,

they have mixed the effusion contained in the aphthous patches of animals affected with aphthous fever with red cells, certainly killed by exposure in the ice-box for forty-eight hours. They have shown that after a certain period of contact the ultravirus present in the liquid has become fixed to the erythrocytes, and with such a firm union, that washing is unable to dissociate it. They have also shown that the same ultravirus may be in the same way adsorbed to any bacterial form whatever, even though they are killed by heat. It is not then an adequate reason, because an ultravirus is found adherent to a certain type of cell (chiefly the formed elements of the blood) to draw the deduction that this cell is the site of election of the parasite.

Viability

The vitality of the ultraviruses is, apparently, markedly variable, in accord with the species. But here we ought first to make a general statement, to which we will have to refer again in connection with a consideration of temperature, namely, that it is really improper to speak of an ultravirus as *destroyed*, for of that we know absolutely nothing. We only know that under certain circumstances it loses its activity, that is, that it loses the property of causing the disease of which it is the causative factor. In brief, it loses its virulence. And that is all that experiment allows us to affirm. An ultravirus is an obligate parasite, and we can only detect its presence when it causes disease; but, whether it be killed or whether it be simply rendered avirulent, the result would be the same, its inoculation would not be attended by disease.

This loss in virulence has, however, a very great practical importance. It is well known that it is very often impossible to discover either the origin of an epidemic, or the origin of the contamination in the sporadic cases of diseases caused by the ultraviruses. But, it is highly probable that the pathogenic ultraviruses may remain alive, although avirulent, for a very long time in the external world or even in certain organisms. In this way Vallée and Carré have shown that the virus of equine pernicious anemia persists for a very long time in the blood of the animal after recovery. It is also clear that certain plants may be the healthy carriers of the mosaic ultravirus, just as Flexner and

Amoss, as well as Kling, have demonstrated that the number of healthy carriers of the virus of poliomyelitis is much greater than the number of persons affected with the disease. But we also know that certain individuals are particularly susceptible to certain diseases (some may never acquire an immunity, against vaccinia, for example) and that an ultravirus avirulent for a normal person may invade the body of such a susceptible person and bring about the disease. The ultravirus may thus enhance its virulence and then be able to parasitize a normal individual.

Having said this much, it is clear that the different ultraviruses *under the particular conditions of experimentation*, may be inactivated by the period of preservation, and that this may be very variable according to the species. Certain of them are very fragile at ordinary temperatures, such as the ultraviruses of rabies, vaccinia, and a number of others. With some others the period of preservation is very long, being several months, or even several years, as is the case for, among others, the mosaics, the bacteriophage, that of sarcoma, and of avian plague. And observe this, which shows the fundamental nature of the observation made on the question of the apparent destruction which may perhaps be simply a loss in virulence, that, for all of them, the virulence diminishes progressively even to the point where a complete inactivity can be shown. The Pasteur method of treating rabies is based, in part, upon this fact. We will speak of this again later.

Some lines back I emphasized "in the particular conditions of experimentation." It must be stated that experiments concerning the period of viability relate for the most part to cases where the ultraviruses are enclosed in a tissue, and this limits the value of such determinations. Is it because of time, or because of the action of the tissue that the inactivation takes place? As regards the virus of rabies, it has been clearly shown that it is the tissue which is operative. It is impossible to determine the true viability of an ultravirus unless it is in suspension in a neutral fluid, which, by itself, does not exert a destructive action. Until such an experiment may be accomplished it is well to state that "under such conditions" the inactivation occurs after such a time, or under such a temperature, since these do not absolutely determine the true vitality, which may be quite different.

At low temperatures, even when the ultravirus is enclosed in a tissue, the period of preservation is far longer than at room temperature, most probably because at the lower temperatures the tissue ferments are unable to act. The same result is obtained by desiccation.

The action of temperature

Let us repeat the above statement, that brought to a certain temperature, the ultraviruses commence by becoming attenuated, and this the more quickly the higher the temperature. Then, at a given temperature they cease to be active. Are they dead? Certainly not, and as proof of this we may cite the bacteriophage, for it is possible to obtain cultures from an emulsion heated to a degree sufficient to cause apparent sterilization. It is only necessary to make several passages with a susceptible bacterium to see the virulence reappear and to gradually increase. One can not therefore speak of the temperature of destruction of an ultravirus, but of the temperature at which it becomes avirulent *under the conditions of the experiment*, for if one is working with a virus surrounded by tissue the effects of the tissue must be added to those of temperature. The true temperature of destruction is most assuredly higher than those which have been suggested up to the present time.

Under the conditions in which the experiments have been carried out the resistances of the different ultraviruses to the effects of temperature have been very variable, according to the species. Many are inactivated at temperatures of approximately 55°, such as rabies, vaccinia, aphthous fever, measles, and encephalitis. That of myxoma of the rabbit loses its virulence at about 60°. The bacteriophage, and the virus of avian plague are more resistant still, for with them inactivation does not take place until at temperatures between 63° and 75°. And finally, the mosaics do not lose their virulence until raised to temperatures of about 100°.

The temperature at which we cease to be able to demonstrate the presence of an ultravirus being that of its inactivation through the loss of virulence, and not that of its destruction, we can understand that this temperature may be different, for a single ultravirus, according to its degree of virulence. An ultravirus

of low virulence may lose its power of infectivity at a temperature far lower than another strain of the same species, which possessed a high virulence. This fact is particularly well demonstrated with the bacteriophage. I have shown that it is possible, by successive passages with a susceptible bacterium, to restore gradually the virulence of a bacteriophage, which had apparently been destroyed by heat. And it is for this reason that I stated above that the temperature of inactivation of the bacteriophage took place between 63° and 75°. A single strain, when its virulence is low, may be inactivated at 63°, but if its virulence is enhanced by successive passages, it is then inactivated only at temperatures approaching 75°.

The following facts may probably be attributed to the same cause. Levaditi has shown that vaccinal lymph is inactivated by exposure to a temperature of 55° for thirty minutes, while at this same temperature the virulence of the virus derived from the brain is not impaired. But this last virus appears to be of higher virulence than the first. As regards the ultravirus of avian plague it is completely inactivated by subjection for thirty minutes to a temperature of 62°, although it may resist a temperature of 68° for four hours if it is derived from the central nervous system (Prowazek).

It appears that the effect of temperature upon the ultraviruses is very complicated. Moreover, it is necessary to state that in so far as the bacteria are concerned, the action of temperature is perhaps not so simple as has appeared. Surely we may safely say that as yet we do not know bacterial physiology, for the simple reason that we have entirely neglected the question. Up to the present only the medical aspect of bacteriology has appeared of interest, and despite the fact that we ought to know better the physiology of the bacteria, bacteriologists grope among these facts without reaching and revealing facts of general importance. The bacteriophage can not be seen, but its properties and its physiology can be studied, and the statement might be ventured that this ultravirus is actually better known than many a visible organism.

The action of antiseptics

The antiseptic having the most remarkable action upon the ultraviruses is glycerol. The majority remain alive for a very long time in 50 to 60 per cent glycerol solutions. Indeed, but little is known of the effect of concentrated glycerol, for it is evident that when a fragment of tissue is introduced into glycerol, it is impossible to speak of a definite concentration of glycerol, for the fluids of the tissue quickly dilute it. Noguchi noted that the virus of vaccinia quickly lost its infecting power in a solution too concentrated. Recent experiments (Remlinger) likewise show that rabies virus is destroyed in a relatively short time in concentrated glycerol although it resists for a long time the action of a 50 per cent solution. It is quite the same for the bacteriophage, it remains active for several months in 50 to 60 per cent glycerol, but is inactivated within six to seven days in a 95 per cent solution (Bablet) and in a few hours in pure glycerol (Proca).

Chloroform, carbolic acid, and ether, are less effective upon the ultraviruses than upon bacteria. Essences kill bacteria quickly but are not markedly active upon the ultraviruses, at least, upon those tested up to the present time, such as the bacteriophage and vaccinia. These two viruses are also resistant to the action of certain dyestuffs, such as fuchsin, crystal violet, and brilliant green. Even toward very potent antiseptics they manifest a high resistance; the ultravirus of avian plague is resistant to antiformin (Hübener) and mercuric chloride (Uhlenhuth).

Certain ultramicrobes are inactivated by bile, those of chicken sarcoma, of encephalitis, of herpes, and of myxoma of the rabbit, while others are unaffected, as is that of poliomyelitis. This action of bile is, however, somewhat dependent upon circumstances for it certainly does not bring about a destruction, but rather an attenuation. A bacteriophage of weak virulence appears to be destroyed by bile, but like the effect of heating, its virulence can be restored and increased by passage. A highly active bacteriophage is unharmed. A comparable condition is observed in the case of avian plague; the ultravirus isolated from the liver of a sick animal is inactivated by saponin, while that derived from the brain is untouched (Prowazek).

All of the ultraviruses are destroyed by the action of alcohol or of 80 per cent acetone. As regards acetone particularly, Peyton Rous has shown that the virus of the infectious sarcoma of chickens is at first precipitated and then destroyed. I have observed the same fact in connection with the bacteriophage. As a matter of fact, it may not be very exact to speak of the precipitation of a virus, for in one case as in the other, the virus is found in a liquid which contains different organic products. That which the acetone precipitates may possibly be those protein substances which surround and carry down with them the ultraviruses.

Both the bacteriophage and the ultravirus of vaccinia are digested by trypsin.

To summarize, one might say that the ultraviruses are, in general, more resistant to the action of antiseptics than are the vegetative forms of bacteria, and less resistant than are the spore forms. This is stated without any idea of establishing a species relationship.

Multiplication

For all ultraviruses multiplication appears to be extremely rapid. The numerical proof of the numbers of an ultravirus in a given quantity of a liquid is indeed a delicate procedure, except in the case of the bacteriophage. With a bacteriophage of maximum virulence acting upon a suspension of dysentery bacilli or staphylococci containing 300 million bacteria per cubic centimeter, after complete lysis, the number of ultramicrobes per cubic centimeter of the liquid may be as high as 10 to 12 thousand million. Comparable figures have been obtained by several authors with respect to the virus of avian plague. According to Belfanti, for example, the blood of a sick chicken is still infective in amounts of a ten-thousand millionth of a cubic centimeter, indicating that here there must be at least ten thousand millions of the ultramicrobes per cubic centimeter of blood.

Variability

All of the ultraviruses manifest to a high degree variability of characteristics, and this is a result of their power of adaptation. This last is far superior to that of other living beings, as might be

expected. For the more simple a being the more plastic it must be, and the greater the distance between the extreme limits of the environmental conditions compatible with life. On the contrary, the more complex the being, the more closely are these limits drawn together. Those beings most readily adaptable, and as a result those which manifest the highest degree of variability in their characteristics, are the ultraviruses, and next to them stand the bacteria. At the other end of the scale are those in which adaptation takes place most slowly, where the characteristics of the species are most firmly fixed, the higher vertebrates.

Later we may return to this highly important question of the variability in the ultraviruses, discussing it somewhat further than we can here.

THE NATURE OF THE ULTRAVIRUSES

When Pasteur discovered the virulent properties of the nervous centres of animals affected with rabies, in the impossibility of distinguishing any bacterium, he turned immediately to the conclusion that the agent of rabies must be a bacterium too small to be perceptible.

For him, who had struggled so hard to make people accept the fact that all fermentation, in the broad sense of the word, was due to a living being, an infectious disease could only be caused by a parasitic living being which provoked a "fermentation" in vivo, and contagion was the resultant of the transmission of this living parasitic being from one individual to another susceptible individual. The fact was for him quite beyond discussion, for he had shown that this parasitic being was susceptible to exaltation and to attenuation, that is, to adaptation, an inherent property of living beings, and, when multiplying at the expense of the cells of the infected animal, it possessed the power of assimilation. It is indeed certain that Pasteur had too little vision to think that the day would come when the living nature of the ultraviruses would be questioned, for with his sound logic, he would never have conceived that anyone could have denied the living nature of a being possessing these properties. To advance such a doubt is to state, in fact, that a being which possesses all of the characteristics of life can not be alive, in other words, it is equiva-

lent to saying that a being can be at the same time, both living and dead.   This seems a rather strange conception!

This discussion began in connection with the ultraviruses of the plant mosaics.   The argument followed this line of reasoning.   It is only the young leaves in which the cells are actively undergoing division which are capable of contracting the disease. The ultravirus develops in the cells, hence the first act of infection must be the penetration of the cell.   And it is readily understandable that the ultravirus may be able to penetrate a young cell, provided with a succulent membrane, and not be able to act upon a cell covered by a membrance of a tough consistency.

This same argument was repeated with respect to different ultraviruses, that of the chicken sarcoma, and that of bacteriophagy among others.

What is the significance of such an argument?   Is the differential character "ability to penetrate a young cell," "inability to penetrate an old cell" a criterion of life?   Shall one say that a being possessed of all the other characters of living beings since it has the powers of assimilation and of adaptation, can not be a living being because it attacks only cells which manifest certain special conditions?   To ask the question is to show the nonsense of such an objection.

But if the ultravirus, the agent of a contagious disease, is not a living being, what is it?   That this substance reproduces can not be denied, nor is it denied by anyone.   But how can it reproduce if it is not a living being?   It might be, as certain authors have said, an "enzyme" formed by the diseased cell. The cell, attacked by the virus-ferment, liberates the same virus-ferment, whence the multiplication in the course of the action and the possibility of contagion.   But then this conclusion is inevitable.   The virus-ferment liberates in the cell a virus-ferment like itself.   The sequel is that the cells of all susceptible organisms contain within themselves all of the virus-ferments of all of the diseases to which they are susceptible.   The cell of the man, for example, contains the virus-ferments of rabies, of mumps, of herpes, of measles, of scarlatina, of poliomyelitis, of encephalitis, of, in a word, all the ultravirus diseases known and as yet unknown, and indeed (a strange contradiction) contains at the same time,

the ultraviruses of vaccinia and of variola. How does it happen then, that a man can contract this small-pox?

The theory of a virus-ferment reduces itself to an absurdity. I know full well the objection which will be made, as Doerr has stated it, that the virus-ferment is not naturally present in the normal cell, but that the virus-ferment which is introduced into the body produces a disorganization of susceptible cells, a vitiation of cellular metabolism, which causes the formation of the virus-ferment. This indeed, is the old theory of Stahl, utilized by Liebig in his discussion with Pasteur of the question of fermentation. It is the theory of "fermentation by communicated motion." "All bodies brought to a state of putrefaction," said Stahl, "very readily transmit this state to another body as yet free from corruption." Today no one would dare to sustain such a theory in support of the fermentations caused by visible bacteria, but the same theory appears again with respect to the ultraviruses.

As has been so often said, history is forever repeating itself, and the history of science appears to be particularly subject to such repetitions. The dead theories must be killed anew. That the struggle of Pasteur should have to be repeated will doubtless prove astonishing to the students of the future. Against the principle formulated by those opposed to the living nature of the ultraviruses, namely, that "all cells in a state of disorganization transmit very readily this state to a cell still free from change" (for this is indeed their postulate) it is necessary to prove once more that the law announced by the clear genius of Claude Bernard, by Pasteur, and by Koch, is always true, that "all disease reproducing in series arises from a living germ capable of multiplying in the body."

There is after all an experimental proof which shows beyond doubt that the cell does not react in any case to produce an autodestroying ferment. In all ultravirus diseases whether it be of the bacteria caused by the bacteriophage, or whether it be of the epithelial cells of a man caused by the virus of variola or whether it be of the nervous cells caused by the virus of rabies, the cell always reacts. But this reaction is directed toward the *destruction* of the pathogenic ultravirus. Experience and experiment show this to be true, and the theory of the virus-ferment, a pure specu-

lation of the mind, having no regard for such facts, is contrary to fact.

I have given the direct proof that the bacteriophagous ultravirus must be a living being because it possesses the "criteria" of life. Experiment likewise shows that all of the other ultraviruses possess to a high degree the power of adaptation, that they are autonomous beings, independent of the cells at the expense of which they reproduce, as is shown by the reaction of complement fixation, that these autonomous beings reproduce at the expense of cells by a process which is analogous to a process of assimilation, that they, also, possess the criteria of life and are necessarily living.

Those who would deny the living nature of the ultraviruses should first answer this question. Do the powers of assimilation and of adaptation constitute criteria of life? If the answer is in the affirmative the subject is closed; if the reply is in the negative can they tell us what the criteria are? To transport the question to any other basis is simply to renew the scholastic discussions of the middle ages, arguments which were considered closed long ago.

THE MODE OF ACTION OF THE ULTRAVIRUSES

The assimilation carried out by the ultraviruses appears to be of the same nature as the assimilation effected by all other living beings, that is, through the intervention of enzymes which degrade the foods into their soluble constituents. As yet the bacteriophage is the only one which allows a study of this question.

The bacteriophage multiplies only at the expense of living bacteria. On the other hand it is well known that certain strains of the bacteriophage have a virulence restricted to only certain bacterial strains, as is particularly the case with the staphylococcus. But, I have shown that a variety v, possessing a virulence strictly limited to a single strain of staphylococcus V, causes the lysis of any strain of the styphylococcus whatever, either aureus or albus, if the bacteriophage is caused to operate in a mixed suspension of the *Staphylococcus aureus* and the susceptible *Staphylococcus albus V*. Such passages may be multiplied without the development of a virulence for *Staphylococcus aureus*.

We must assume then, that the mode of action is the following. The bacteriophage multiplies at the expense of the albus strain V, it produces a lytic enzyme which brings about the dissolution of this staphylococcus, but this enzyme is distributed through the medium and likewise dissolves the aureus strain. The bacteriophage does not parasitize it directly.

Here is another fact. The bacteriophage can develop only at the expense of living bacteria, but Gratia (the first to show this) demonstrated that it is able to dissolve after a very long time, several weeks, a suspension of dead bacteria. I should add that I have shown, with the staphylococcus, that the bacterial bodies contained in 10 cc. of a suspension of staphylococci containing 200 millions of the organisms per cubic centimeter and killed by heating may be completely dissolved at 38° within the space of four days under the effect of 0.1 cc. of a fresh unfiltered culture of the bacteriophage. No multiplication of the ultravirus is observed.

I have also shown that a mono-virulent culture of the bacteriophage, acts under these conditions upon killed staphylococci belonging to a strain for which when alive, the bacteriophage possesses no virulence. Here again the bacteriophage does not reproduce, serial action does not take place.

This dissolution is not the result of the fact that the bacteriophage is a living being. It is the lytic ferment, always present in cultures of the bacteriophage which is active. In effect, a culture of the bacteriophage is a suspension of the ultravirus in a liquid in which bacteriophagy takes place, hence this liquid contains not only the substances secreted by the bacterium prior to its dissolution, but the ultravirus, and the bacterial substances rendered soluble by its action, and finally, the products secreted by the bacteriophage itself. It can only be the last of these which is operative upon dead bacteria.

It is then probable that with all ultraviruses the act of assimilation is accomplished by a mechanism identical with that obtaining in all other living beings.

The protoplasmic substance of the ultraviruses differs from the substance of the beings at the expense of which they multiply.

Proof of this is provided by complement fixation which shows that the antigenic ultravirus is different from the antigenic substance of the being which is parasitized.

Furthermore, the substance constituting the ultraviruses is homologous for all of the ultraviruses belonging to a given species. For vaccinia virus, for example, the complement fixation reaction shows that the substance of all strains of the vaccinia ultravirus is identical, whether it multiplies at the expense of a human cell, of a cell of the rabbit, or the cell of the cow. In the same way the substance of the bacteriophage is the same, whatever may be the bacterium at the expense of which it is reproduced; whether it be the staphylococcus, the cholera vibrio, the dysentery bacillus or the plague bacillus, for all of the bacteriophages function as a common antigen.

Thus from the point of view of assimilation and from that of the constancy of protoplasmic composition, the ultraviruses differ in no way from the situation encountered in beings much more highly organized. We have seen also that it is the same from the point of view of adaptation. This, however, could be predicted, for assimilation and adaptation are criteria of life, and the intimate processes of these actions ought to be the same for all living beings.

CLASSIFICATION OF THE ULTRAVIRUSES

Ultrafiltration experiments show that the ultraviruses are formed of particles having a diameter of about 20 millimicrons, a diameter approximately equal to that of the albuminous micella. Consequently these beings can be formed of but a single protoplasmic micella. They are elementary beings, the most simple of all living beings, and it is for this reason that I have proposed for this group of *unimicellar* beings the generic term of "Protobios," a group placed at the very bottom of the animal and vegetable kingdoms.

In view of their dimensions and of their comparable properties, they ought, most probably to be included within a single genus, the genus Protobios.

The ultraviruses, sensu stricto, that is to say, those whose

diameter has been determined and which are most certainly unimicellar would then be:

Protobios bacteriophagus (syn. *Bacteriophagum intestinale*, d'Herelle, 1918) parasitic of bacteria

Protobios mosaicus, the cause of plant mosaics. Of this there are several varieties, the type being *Protobios mosaicus*, var. *tabaci*

Protobios pestiavis, agents of avian plague. Type: *Protobios pestiavis*, var. *gallinae*, the cause of the disease in the chicken

Protobios variolae, cause of different human and animal diseases. Type: *Protobios variolae*, var. *bovis*, the cause of cow-pox or vaccinia

Other varieties are: *hominis*, the cause of human variola

ovis, the cause of sheep-pox

equi, the cause of horse-pox

sui, the cause of variola in swine

Protobios lyssae, the cause of rabies, with most probably different varieties, such as the pseudo-rabies of cattle, for example

Protobios variabilis, causes of different diseases. Type: the cause of human encephalitis lethargica

Probable varieties may include:

influenzae, cause of influenza

herpeti, cause of herpes

varicella, cause of human varicella

It is certain that the majority of the filtrable viruses actually known will be classed in the genus Protobios just as fast as ultrafiltration experiments show that they are formed of a single protoplasmic micella.

A word might be added upon the question of "species." Species result from a differentiation accomplished within a given "genus," just as the genera result from a differentiation within the type "family." Variability within a being is a function of its degree of complexity, the more elementary the being, the greater is its faculty of adaptation, and conversely, the more complicated a being, the higher it is in the scale of organization, the less pronounced is this faculty.

The idea of family, of genus, and of species, has a definite meaning among the vertebrates. But the lower one descends in the scale of beings, the more the genera and the species multiply, and the conception of a species becomes less precise. With the ultraviruses, elementary beings, the variability is such that the concept of species loses all significance. One can only speak of varieties which are not fixed. By adaptation an ultravirus of a

given variety may quickly pass over into another variety. We have seen an example of this with respect to the bacteriophage, and we will see in the next chapter that it is the same for the other ultraviruses.

THE ORIGIN OF LIFE

The probable origin of life is a subject of wide discussion. For some (of whom Osborne is an example) the primitive being must have been a bacterium, for others (represented by Costantin) it should be a cell provided with cholorophyll, and for Buchanan it must have been one of the Thiobacteria, organisms which are able to live in a purely mineral medium, utilizing as sources of carbon the carbonic acid which they make, thanks to the energy which they develop in the reduction of sulfuretted compounds.

This last conception appears by far the most probable, but not in the form given, for the primitive being is certainly the most elementary one, the ultravirus.

I have conducted some experiments with sulfur-containing water, the waters of Challes-les-Bains (France) with the curious results here presented.

I have inoculated water filtered through an *ultrafilter* (permitting the albumin micella to pass through) into the medium devised by Beijerinck for the cultivation of the Thiobacteria[9] and on several attempts I have secured cultures of Thiobacteria, in some experiments of a single species, in others, of two different species. The experiment succeeds particularly well if the amount of the ultrafiltered water is large, 2 to 3 cc. These Thiobacteria possess, then, ultrafiltrable forms.

But here are further data. In the course of the preceding experiments, 17 times the medium remained limpid when other tubes gave a culture of the Thiobacteria. Of these 17 tubes, 15 remained as they were, indefinitely sterile. The other 2 slowly became clouded after about ten days, and examination showed that the turbidity was due to reduced colloidal sulfur and it was impossible

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[9] Sodium hyposulfite.................................   0.5   gram
Sodium bicarbonate................................   0.1   gram
Potassium phosphate...............................   0.02 gram
Ammonium chloride.................................   0.01 gram
Magnesium chloride................................   0.01 gram
Water...   100   cc.

to demonstrate any visible bacteria, even after a month. The reduction of the sulfur could only have been produced by an ultravirus; this is the only hypothesis possible.[10] This ultravirus may possibly be a common ancestor for all living beings, and might be designated *Protobios protobios*.[11]

However this may be, the ultraviruses form most probably a group of unimicellar beings from which, by evolutionary processes, all other living beings have developed.

There has been much discussion, and this argument continues, upon the question of the nucleus in bacteria. A nucleus is not demonstrable. Always under the obsession of the preconceived idea that a living being must necessarily be cellular, that is, constituted of a differentiated protoplasm many scientists have sought desperately for this nucleus. Schaudinn was unable to find it, and, despite his remark that the nucleus can only be defined morphologically, had advanced the hypothesis of the existence of a "diffuse nucleus." This is a singular supposition. Since it is impossible to demonstrate that a nucleus exists, although on the basis of theory it must exist, it has been assumed to be "diffuse." As a matter of fact, if we would be guided by these facts, this discussion must be unnecessary, for in consideration of the experimental evidence, the bacteria (aside from certain forms closely related to the Cyanophyceae) could not be cellular beings. What then can they be?

I have observed the curious fact[12] which I have not been able to explain, that it often happens that a bacterial culture dissolved through the agency of the bacteriophage, and filtered through a bougie, again becomes turbid, and that it can be shown that this turbidity is due to a culture of resistant bacteria. Tomaselli has undertaken the study of this phenomenon and has seen that it

[10] Needless to say controls were always made, and for greater surety I have inoculated the same medium with the sulfur-containing water passed through very close ultrafilters, such as did not permit the passage of either the colloidal albumin micella or the bacteriophage. All tubes inoculated with this material remained sterile indefinitely.

[11] It is curious that no one has ever investigated that which can only be an organic substance, non-organized, termed "barregine" which is contained in many sulfur waters as they come from the ground.

[12] Mentioned in *The Bacteriophage, Its Rôle In Immunity.*

occurred almost always, when the bacteriophage with which he was working was of low virulence.

One thing is sure, that there occurs under the conditions of the experiment filtrable forms of the bacteria.

I have indicated above that the ultrafiltrates of sulfur waters may yield cultures of the thiobacteria. Here again, are bacteria which, normally this time, possess filtrable forms, and these filtrable forms are micellae.[13] In this connection mention may be made of the following rather curious fact. When injected into the tissues of a plant *B. tumefaciens* causes the development of a tumor. If fragments of this tumor are seeded into an appropriate culture medium a culture of the bacillus is obtained, despite the fact that the direct examination of slides prepared from the tumor itself never reveals the presence of any visible bacteria whatever. The explanation is, perhaps, that in the artificial medium the bacteria develop in the form of micellar plasmodia, the ordinary bacterial form, while in the parasitic stage the form is unimicellar. This must be, then, the form as it multiplies in the vegetable cell: in effect, an ultravirus.

It appears necessary to conclude that, as I believe to be the case, the bacteria are *plurimicellar* beings, but that under certain conditions, they may develop in the unimicellar form, and be reconstituted by *aggregation* into plurimicellar bacteria. The bacteria may be micellar "plasmodia."

Bacteria may be the first link in the chain of the vegetable kingdom. How does the nucleus make its appearance? Perhaps as a result of a symbiosis, a process so frequently taking place in nature that it forms truly a general phenomenon, as has been shown by Noël Bernard. It may be rather as a result of a simple physico-chemical process since between the nucleus and the protoplasm the differences are quantitative and not qualitative.

The group of Protobes may be the source, on the one hand of the bacteria, of vegetable nature, and on the other of the Protozoa the first stage in the animal kingdom.

[13] Several authors have demonstrated that certain bacteria have filtrable forms (*B. tuberculosis*, Vaudremer) and even ultrafiltrable forms (Heymans), and this normally, in the absence of the process of bacteriophagy.

To express the relationship, the successive development of living beings may be schematically represented in the following manner:

Unimicellar beings
(Ultraviruses—Protobes)

Plurimicellar beings
(Bacteria)
||
Cellular beings
(Fungi)
||
Pluricellular beings
(Vegetables)

Plurimicellar beings
(Undifferentiated protozoa—Spirochetes)
||
Cellular beings
(Differentiated Protozoa)
||
Pluricellular beings
(Animals)

PROTOBIOLOGY

The ultravirus, or Protobe, which represents the most rudimentary being possessing elementary life, is nevertheless endowed with the same "powers" as beings much higher in the scale of organization.

The true ultimate end of the physical sciences, is the recognition of the intimate nature of matter, and physicists have made great advances in these studies during the past few years. But, let us state it is not by studying matter in its tangible form that they have advanced, but by scrutinizing invisible matter.

They have shown us the path to follow.

For us, as biologists, the ultimate problem is the determination of the intimate nature of life. In order to solve it, we can not concern ourselves with the visible organisms, for in them the complexity of the vital phenomena is too great, too many factors intervene which hinder us. That which we must study is elementary life, formed of the single protoplasmic micella in which the simplicity of the phenomena is greatest.

To be hypnotized by form represents a regression to an epoch already remote where morphology represented all science. It is to return to the errors which took place during the reign of scholastic philosophy, a philosophy of which the last vestiges are to be found in the cellular conception of life.

Today the object of all research is not to "see" but to "know"

properties. Sight is certainly the sense which most frequently leads man into error, for man has an instinctive faith in seeing. He will lose this faith only when he submits to reason the facts which science reveals to him. It is possible to thus boldly announce the principle that sight only leads to error and that it is only when science frees itself from the idea of seeing that it will attain the truth, since as evidence we have only to consider the old ideas of astronomy or the more modern conceptions of physics.

One can not see the ultravirus, perhaps they never will be seen, but it is possible to study their properties, and by these to understand them completely. If we will ever see the time when we shall know the nature of the origin of life, it is certainly because the study of the ultraviruses, Protobiology, will reveal it to us.

ULTRAVIRUS DISEASES

We have seen in the preceding chapter that the question of the ultraviruses is burdened with confusion. The term "filtrable microbe" is in itself an ambiguous expression. The cause of pleuropneumonia in cattle is a filtrable microbe, but in reality it is a minute bacterium capable of being stained and observed under the microscope. The agent of yellow fever is filtrable, possessing, most probably, an evolutionary cycle of which a part is passed within the body of an insect. The cause of rabies is also filtrable, and this can be nothing more than a single living protoplasmic micella. Each of these three types can not be compared directly with the others, and there is no reason whatever why the immunity against three so dissimilar beings should be the same. This is the more true since we will see that the defensive reactions of the body against an ultravirus are in the strict sense of the word comparable to none of those processes caused by visible bacteria. The immunity to the ultraviruses is a specialized immunity, and it is for this reason that I have chosen to term it "antivirus immunity," the principle resulting from the reaction of the organism being designated by the word "antivirulin."

Inexactness is a very great obstacle to the advancement of a science, thus, in order to define the disease caused by an ultravirus it is essential to first state again just what is meant by an ultravirus. Ultraviruses are exclusively those autonomous beings which are alive, because they possess the powers of assimilation and of adaptation, and which the ultrafiltration experiment shows to be of the order of size of the protein micella. An ultravirus is, in short, a living unimicellar being.

In the discussion to follow we will consider, then, only the mode of reaction of the body when parasitized by an ultravirus in the strict sense. (The mode of reaction against a filtrable bac-

terium, or of a protozoan having a filtrable form, belongs in general to the endogenous immunity already discussed.)   The only ones which have been determined as such up to the present are, the ultraviruses of bacteriophagy, the mosaics of plants, those of avian plague, of rabies, of vaccinia, and of encephalitis.   For a certain number of others, although the final proof of ultrafiltration has not yet been provided, we can be very sure that they will be included with the true ultraviruses because of analogies between the diseases which they cause and those having a known ultravirus as the causative agent.   Such are the agents of alastrim, of human variola, of sheep-pox, and of variola of swine.   These are certainly of the same nature as that of vaccinia.   The agents of herpes, of varicella, of zona, and probably of influenza present certain analogies with that of encephalitis.   The agents of the diverse animal plagues are, because of the similar characteristics of these diseases, certainly caused by viruses resembling that of avian plague.   And further, if we also consider the behavior of the pathogenic agents toward antiseptics, in connection with the type of immunity conferred by the disease, we see that it is very probable that all of the agents of the directly transmitted diseases enumerated at the end of the preceding chapter must belong to the ultraviruses.

THE INCUBATION PERIOD

In diseases due to ultraviruses, the incubation period is very variable in accordance with the disease.   Often it is very short, two to three days in the case of vaccinia and variola, and for others it may be very long, even several months.   We should state, however, that for most of them we have only presumptive evidence based upon epidemiological studies.   In a few cases the period of incubation has been determined with certainty, because of the fact of the particular mode of infection.   In rabies, the minimum incubation period is, in the case of man, about ten days.   The average period is between thirty and sixty days, but in some cases it has certainly been shown that the period may be very much longer (for man, eight months, for animals, fifteen months).   In contagious epithelioma of birds, or molluscum, the disease appears only several months after the inoculation (Lipschütz), and the

same is true for verruca in man, which does not appear at times until after several months from the time of inoculation (eleven months, Frey).

On the other hand we know that certain of the ultraviruses are able to maintain themselves in the body in a "latent" state without causing the organism which harbors them to show the least disturbance. This is true for the bacteriophagous ultravirus. Certain bacterial strains, rather rare, however, maintained as laboratory stock cultures contain the bacteriophage in a latent condition. These bacteria are in reality "healthy carriers." But under certain conditions of diminished resistance on the part of the bacterium the disease, bacteriophagy, breaks forth.

In a similar way, as is well known, some plants, because of climatic conditions, may remain indefinitely "healthy carriers" of the mosaic ultravirus.

Observations of the same general nature have recently been recorded by Netter and Urbain.[1]

In 1865 Leudet noted that carbon monoxide intoxication frequently was followed by zona. Shortly afterward, in 1868, Hutchinson showed the rôle of arsenic in the pathology of zona, basing his observation upon several cases of the disease following arsenical therapy. As particularly characteristic we might cite the observation made by Reynolds in 1899. A peculiar epidemic of polyneuritis occurred in Manchester among those who drank large amounts of beer, and the condition was first attributed to the alcohol. Reynolds was impressed by the abnormal frequency of of zona in these cases, and suspecting an arsenical intoxication, he demonstrated that the beer contained arsenic as the result of the use of impure glucose by the brewers.

The use of salvarsan, or neosalvarsan, and more recently of bismuth compounds (Lehner) in the therapy of syphilis has frequently been assigned as the cause of zona.

But, Netter and Urbain have demonstrated that these cases of zona associated with arsenic and bismuth treatment are in reality caused by the ultravirus of herpes, or rather by the herpes-zona-varicella virus. We must from this conclude that this ultravirus is present in a latent condition in a large number of persons who are

[1] Compt. rend. Soc. de biol., 1924, **90**, 997.

in effect "healthy carriers." The ultravirus attacks the susceptible cell only when the latter is subjected to particular conditions, otherwise the virus remains indefinitely latent.

This suggests a thought with reference to cancer. It is well recognized that many agencies which cause irritation may lead to the development of a tumor. We might mention such well-known things as the frequency of cancer among mill workers who come into contact with tar, and the cancer of chimney-sweeps. The processes may be reproduced experimentally, for if mice are subjected to the repeated application of tar nearly all of the animals will develop a carcinoma.[2] The majority of authors have come to the opinion that cancer is not of infectious origin but that it is rather the result of a disturbance in the cellular metabolism due to the irritation. At first sight, it appears that this must be the case. But the facts noted with reference to zona suggest another hypothesis, perhaps equally plausible, namely that the cancer virus may be a normal banal parasite of all organisms, incapable of attacking the normal cell but able to parasitize a cell when certain particular conditions of diminished resistance occur. This idea receives some support from these facts already known, that the malignant tumors of plants are of parasitic nature, that Peyton Rous has shown chicken sarcoma to be due to an ultravirus, and that we know further, that when a cell is attacked by an ultravirus its primary reaction is *always* a proliferative process.

But, it may be said, these facts might also indicate that the ultravirus is a cellular product, the result of an impaired cellular metabolism, brought about through the action of some irritant. Such an objection fails to take cognizance of the fact that we know of many instances of "healthy carriers" of pathogenic bacteria which are most assuredly living beings. And this case presents much in the way of analogy.

Here is another case, of a similar nature, where the hypothesis of cellular origin can not be held valid. For a long time we have

[2] An hypothesis, rather plausible, has been advanced to the effect that it is not the tar as such which is responsible but the arsenic which the tar always contains. This is suggestive, too, in relation to what has been said of zona.

known that the hemaotozoan of malaria may persist in a latent state for many years in an individual. Cases are on record where this condition has persisted for more than thirty years without the development of the disease until a break in the resistance of the body took place. It is of interest that since 1921 many observations have been recorded, particularly in Germany of cases in whom the injection of salvarsan has been followed by malarial attacks, sometimes fatal, in subjects who, formerly, had never had an attack of the disease. Examination of the blood of these persons showed many of the spherical and crescent-shaped bodies characteristic of *Plasmodium praecox*. The arsenic awakened the latent plasmodia.

We must conclude that the majority of persons who have dwelt in malarial districts (and in fact there is hardly a country which is not so) even if they have not had an obvious attack of the disease remain for the rest of their lives carriers of the hematozoa in a latent form.

The case is analogous to that of the bacterium bearing a latent bacteriophage; to that of the plant carrying the latent mosaic ultravirus; to that of the man contaminated with a latent herpes ultravirus; and, probably, to that of an individual who harbors the cancer virus in a latent condition. Such cases are, furthermore, to be considered from the viewpoint of what we know of symbiosis, that phenomenon which we know to be so widespread in nature.

The single difference is in that the hematozoan, or the bacterium, of the healthy carriers are visible and consequently cannot be disregarded, while the ultraviruses, being invisible, can be subjected to doubt. It should be emphasized that the designation "latent state of life" does not mean "destitute of life," and that size leading to visibility has nothing to do with the nature of a being. The cell never reacts by the production of a suicidal principle but by the elaboration of an inactivating principle. We have many proofs of this.

THE CELLS ATTACKED

All of the ultraviruses appear to be intracellular parasites. Some of them remain strictly localized in the tissues containing the

susceptible cells (rabies, poliomyelitis), but the majority may be found in greater or less abundance in the circulation. We should observe that the presence in the blood by no means implies that the ultravirus multiplies at the expense of the cells of the blood, for several authors have proved that the ultraviruses are readily adsorbed by the blood cells, and it may be that even if an ultravirus develops exclusively in the fixed cells and only passes into the blood secondarily that it may be found adsorbed by the circulating cells. This is true of avian plague in the duck and the goose, in which it has been shown that the susceptible cells are in the nervous system, and it is the same with the chicken, but in this last animal in addition the virus is found very abundantly in the blood. Does it grow there directly, or is it derived from the nervous cells? As yet it can not be definitely said, but the second hypothesis is the more probable.

One might conclude from the observations made up to the present time that the cell which has the maximum receptivity for the ultraviruses is the nerve cell, following this, the epithelial cell, and then the germinative cell. The other cells are only rarely attacked, at least in a definite manner, and only by certain ultraviruses, such as that of chicken sarcoma which electively parasitizes the connective tissue cell.

Borrel coined the word epithelioses to designate a group of diseases due to filtrable viruses, comprising variola, vaccinia, sheep-pox, molluscum of man, and contagious epithelioma of birds. Menze attributed the elective affinity of the visible microbes, on the one hand, for the tissues derived from the mesoderm and of the ultraviruses, on the other hand, for the tissues derived from the ectoderm, as being determined by the embryological origin of these tissues. From his researches on herpes, on encephalitis on rabies, on poliomyelitis, on vaccinia, Levaditi has created the term neurotropic ectodermoses for that group of diseases of which the agents manifest at the same time an elective affinity for the epithelial cell and for the nervous cell. This affinity is, in his mind also, determined by the ectodermal origin of the two groups of cells.

Levaditi attributes such a rôle to embryological origin, and, confronted with an intense localization of the vaccinia virus in the

adrenals of the rabbit which had received an intravenous injection of this virus, he said "The ectodermal nervous tissue origin of the medullary substance of the adrenals, will explain the affinity of the vaccinal germ for this organ."

But this fact agrees rather poorly with such theories; if one injects into the circulation of the rabbit some vaccinia virus, the organs which fix it most energetically, those where the growth takes place most abundantly, those which manifest subjectively the most characteristic lesions, are the reproductive organs, in the male the testicle, in the female, the ovary. Even "in an animal which has acquired infection by contact the vaccinia virus may be recovered from the seminal gland." "From this it appears that the affinity of the virus for the elements which enter into the constitution of the reproductive organs, testicles and ovary, manifests itself whatever may be the avenue of the penetration of the germ" (Levaditi). The true elective cell, is then, in the case of vaccinia virus, the germinative cell. And this electivity reaches even to the fertilized ovum and the embryo to which this ovum gives birth (Levaditi).

But, the germinative cells are derived from the endoderm. This alone suffices to ruin the theory which tries to explain the localization of ultraviruses upon the basis of an affinity determined by embryological origin. To say that such an exception but confirms the rule may be perhaps all right for grammarians, but it can hardly be employed wisely in science.[3]

As a matter of fact the diseases caused by visible bacteria are no more "mesodermoses" than the disease due to ultraviruses are "ectodermoses" or "neurotropic ectodermoses." All that can be said is that the visible bacteria multiply in the fluids while the ultraviruses multiply in the cells. Some bacteria, it is true, are to be found in the leucocytes (gonococci, staphylococci, and streptococci, among others) but this does not represent an affinity of the bacteria for the white cell but rather an affinity of the leucocyte for the bacterium which grows normally in the fluids. There are a few visible bacteria which are able by their own

[3] Except that perhaps the germinative cell, according to a conception presented in the first part of this text, must be constituted of an union of the micellar types which comprise the individual.

efforts to penetrate a cell, such as *Treponema pallidum* which may at times be found within nervous cells.

If it is desired to group under a generic name the diseases caused by the visible bacteria, they ought to be called "humoral infectious diseases," while, in contrast, the diseases due to the ultraviruses should be termed "cellular infectious diseases."

VARIABILITY OF THE ULTRAVIRUSES

The extreme variability of the ultraviruses still further complicates the question of specific localization, and in addition, the questions of etiology and of immunity of the diseases caused by these agents.

The ultraviruses, elementary beings as they are, adapt themselves with great ease to the different conditions in which they may develop and may thus acquire new properties. The result is a great variability among the different strains capable of infecting a susceptible individual. However, a number of comparable facts are recognized, although less evident, among the bacteria, but for the latter, since they are more highly developed, the adaptation is much more slow in maturing.

For some years it has been known that the ultravirus of variola, in the course of its development in the epithelial cells of cattle, acquires new properties; being transformed into the vaccinia virus. Alastrim, a benign disease resembling in symptomatology an attenuated variola, is undoubtedly caused by a more or less modified variola virus. In some epidemics of alastrim, the disease immunizes against vaccinia (Leake, Carini), and in others no such immunity is to be observed (Beaurepaire-Arago, Azevedo).

Pasteur has shown that the ultravirus of rabies by passage through the nervous cells of the rabbit, is attenuated for man and for other susceptible animals, while it is exalted for the rabbit. It is well known that the Pasteur method of treatment for rabies is based upon this attenuation. We profit by the long period of incubation to effect vaccination of the individual by intensive injections of the attenuated virus.

The passage of the fixed rabies virus, that is to say, of that adapted to the rabbit, through the nerve cells of the chick attenu-

ates it for the rabbit to such a degree that after only two passages it no longer kills this animal, but vaccinates it. After four passages through the chick it no longer has a vaccinating action, being attenuated to such a degree.

Here are some additional and new facts, although still more characteristic.

The bacteriophage is the ultravirus most readily accessible to the investigator. It may be varied by the infinity of the conditions in which it develops. It can be shown that each individual strain isolated has its own peculiar properties, both as to the intensity of its virulence for a single bacterial species and as to the extent of the virulence for different species of bacteria. In fact, no two strains of the bacteriophage possessing exactly the same properties exist. The bacteria resist the action of the bacteriophagous ultravirus, they may acquire an immunity to the strain to which they have resisted, but still remain susceptible to the action of another strain from another source.

For another ultravirus, that of aphthous fever, we have exactly comparable facts. The virulence of different strains of the aphthous virus is materially variable for a single species of animal, cattle. In the course of certain epizootics the mortality is nil and the disease is benign in all cases, amounting usually to but a simple temperature elevation lasting two or three days and the appearance of a few buccal vesicles. In more severe cases due to this strain vesicles of the hoofs appear which heal quickly without treatment, and pregnant cows do not abort. I witnessed such an epizootic in Indo-China in 1920 and it was possible to follow the very slow progress of the disease, since at this time I had many experimental buffaloes and cattle which were affected. On the other hand, despite its mildness, the disease was extremely contagious, not a single animal in the district of Saïgon escaping the infection.

In other epizootics aphthous fever may be, on the contrary, characterized by a very high mortality. Some animals die then in a very striking manner. The ultravirus of aphthous fever multiplies generally in the epithelial cells, but it may parasitize the nervous cells, and this is what takes place in the latter type of epizootic.

In the serious epizootics, even in the animals which survive, the lesions are marked, the healing of the lesions is protracted, and abortion is a regular occurrence of pregnant animals.

Between these two extreme types of the epizootic, all intermediate types may be observed.

It has been known for a long time that, after certain epizootics of aphthous fever, the affected animals acquire an immunity which protects them from a new attack for a considerable period. In others, on the contrary, the recovered animals do not appear to have any immunity at all, for relapses are very frequent, and may be multiple, occurring at intervals of a few weeks.

Vallée and Carré have recently given the explanation of such an anomalous condition. The aphthous virus is capable of great variability. Experiment shows that a recovered animal possesses a solid immunity against the virus, the agent of the disease. Since this virus can be preserved in vitro for several months, it can be shown that the recovered animal, reinoculated with massive doses of the virus taken during the disease, manifests no disturbance, while a normal cow contracts the disease after inoculation of a dose a thousand times smaller. On the contrary, this animal so solidly immunized does not have any immunity against an aphthous virus derived from sick animals found in another region.

One can understand then the anomalies mentioned above. If an aphthous virus of an epizootic spreading through a region is imported into another region where at that time there is an epizootic, the recovered animals do not possess any immunity against this new virus and a second epizootic is superimposed upon the first. Several epizootics of aphthous fever may thus coexist in the same district, none of them having an immunizing effect for the others.

This absence of an immunity in the recovered animal for a virus belonging to another strain, is not, however, related as one might think to a simple question of virulence. One might, in fact, think that a disease caused by an attenuated virus might not vaccinate against a disease caused by virus of very high virulence, but the variation is more fundamental than this. It is not solely a question of virulence as the following indicates.

Vallée and Carré showed that cattle which had contracted aphthous fever in the neighborhood of Paris, and which experi-

ment had shown to be thoroughly immunized, readily contracted the disease when they were inoculated with a virus derived from an epizootic in Germany. And the reverse was equally true, for these authors showed likewise that animals recovered from the German epizootic, and completely refractory to the German strain, readily contracted the disease when they were inoculated with the virus of the strain infecting the Parisian district. This crossed test shows that the variability is not associated with the question of virulence, for if that had been the case, certainly one of the two strains would have vaccinated against the other.

The variability also extends to the species of animals attacked. In the course of certain epizootics of aphthous fever some animals other than cattle are attacked, for example, sheep, goats, swine, and at times by accident man. But in other epizootics the disease remains confined to cattle. Lebailly, in France, inoculated a man with the aphthous virus and caused no disturbance. Pancera, in Italy, inoculated a cow with the fluid derived from human aphthous vesicles and caused a typical case of aphthous fever.

It is probable that all of the facts mentioned with respect to aphthous fever also play a rôle in human influenza explaining in some measure the many contradictory observations.

Here are other facts which show that the variation may be still more pronounced, to such a point even that a single ultravirus seems to be able to cause absolutely different diseases. This is due, certainly, to the results of an adaptation to parasitism for such and such a species of cell.

The investigations of Doerr, of Levaditi, and of Blanc, have shown the close relationship of the virus of herpes to that of encephalitis. Netter has recently shown that a similar relationship exists between the virus of herpes and those of zona and of varicella.

There is then, on the one hand, a single disease, aphthous fever, caused by different strains of a single ultravirus, and on the other, very dissimilar diseases caused by different strains of the same ultravirus. It is even possible that influenza may belong in the group of diseases caused by an ultravirus of the encephalitis type.

The extreme variability of the ultraviruses retains still, without doubt, many etiological surprises, and from the facts already

known, it is possible to deduce that the number of distinct "species" of ultraviruses must be rather small.

THE CONDITIONS OF CELLULAR INFECTION

The cells most frequently parasitized by the ultraviruses are then the nervous cells, the epithelial cells, and the germinal cells. To what may we ascribe these localizations?

The first act of infection is necessarily the approach of the ultravirus to the cell. The first act is assuredly under the control of chemotropism, which itself is dependent upon the phenomena of surface tension, being in turn reduced to the intensities of the electrostatic charges possessed on the one hand by the protoplasmic micellae which are the ultravirus, and on the other by the cells. This is all that can be said at the present time with certainty.

The fact that an ultravirus is able to fix itself electively is experimentally demonstrable in the case of the bacteriophage. If one introduces this ultravirus, virulent for *B. dysenteriae*, into two suspensions, one of *B. dysenteriae*, and one of *V. cholerae*, for example, and if one centrifuges these two suspensions after thirty minutes, it will be seen that all of the virus has in the one case fixed itself to the dysentery organisms, and in the other that there has been no fixation whatever. If, on the contrary, the experiment is repeated by the inoculation of a *V. cholerae* bacteriophage into the two suspensions, it will be seen that it has been fixed by the vibrios only. The virulence of the bacteriophage for this or that bacterial species is, therefore, associated with a phenomenon of positive chemotropism.

These experiments allow us to see at once that elective fixation is a real fact, and on the other hand, that the virulence for a species of cell, is a function of the electivity of this fixation. We will see that it is the same for other ultraviruses; that of rabies, among others.

The second act of infection is the penetration of the ultravirus into the cell for which there is a positive chemotropism. Here experiment shows that this penetration is effected or is not effected according to the conditions of the cell at the moment. Again, these conditions are determinable in the case of the bacteriophage. With regard to the cell, the bacterium, its critical moment

is at the time of division. The younger the cell the more readily is it penetrated. It is not, however, because the bacterium divides that penetration takes place, for bacteriophagy can take place with bacteria which are certainly not in the state of division, but in these cases the bacterial disease develops much more slowly. The important point is the condition of youth.

This same fact is encountered in the case of the other ultraviruses. The mosaics of plants show that the virus penetrates only the young leaves, that is, those leaves in which the cells are dividing, undergoing frequent rejuvenation.

If a rabbit is injected intravenously with vaccinia virus cutaneous pustules do not as a rule develop. But, as Calmette and Guérin have shown, if, at the moment of injection, the hair is removed from a portion of the skin, many pustules form in this area, and in this area only. Levaditi has shown that the removal of a hair leads to "a proliferative process which involves the Malpighian layer and especially the epithelial stratum of the hair bulb. There is a very marked karyokinetic division of the epithelium." The conclusion is this, that in the presence of the virus of vaccinia, the critical moment for the epithelial cell, is the moment of mitotic division. Furthermore in the process of Jennerian vaccination the ultravirus of vaccinia is only able to infect the epithelial cell because there occurs a regenerative cellular process as a result of the traumatism caused by the inoculation.

If one injects into the circulation of a chicken the sarcomatous virus no lesions ever appear. But if one takes care (Pentimali) to produce, at the time of the inoculation, a burn of any muscle whatever, leading thus to a regenerative process with cellular proliferation, a tumor results. In the same way, if one injects into a muscle a suspension of the sarcomatous ultravirus, that is to say, the filtrate of a tumor emulsion, neoplasms never result, but they do develop if infusorial earth is added to the suspension, since this causes a proliferation of the cells (Rous).

Here are then, four ultraviruses, attacking cells of quite different kinds, which show the same peculiarity of attacking electively only young cells. What is the reason for this fact? The idea immediately enters the mind, that the old cells present some obstacle which is not present in the young cell. In the case of the

mosaics this is obvious.   The vegetable cell possesses a membrane
in the true sense of the word, in the young cell this membrane is
succulent, later it gradually toughens.   One can conceive perfectly
that an ultravirus might enter the first and not be able to pass
through the layer when toughened.   The case is almost comparable
in the epithelial cells, which, with old age, become keratinized.
For the bacteriophage, the peripheral layer of the bacterium
has such a significance as regards the penetration of the parasite,
that the susceptible bacterium becomes refractory precisely
because it surrounds itself with a mucous capsule which impedes
penetration.

Is the penetration of the ultravirus brought about actively or
passively?   In other words, does the ultravirus secrete a dissolv-
ing enzyme or is the question reduced to a chemotropism between
the virus and the contents of the cell?   This can not be answered
in a final manner, although the following experiment seems to show
that the first hypothesis is the true one.   If one takes a drop
of a bacterial suspension at the moment when bacteriophagy is
taking place, and if one spreads this drop on a cover-glass and then
presses it firmly upon a slide, it can be shown that the contents of
the parasitized bacteria come out of the cell, through one or through
several openings.   These openings can only be the points of
penetration of the parasitic ultraviruses, orifices certainly much
larger than the size of the parasite itself.   This indicates that there
must have been a corrosion of the walls.

However this may be, we see that the affinity of the ultravirus
for a cell is determined by a phenomenon of chemotropism, this
positive chemotropic force representing the virulence of the
ultravirus for the cell against which it manifests itself, and in the
second place, the chemotropism being positive, the cell is, or is not,
parasitized according to the conditions of the cell at the particular
time.

THE INITIAL CELLULAR REACTION

One of the fundamental properties of living matter is the capacity
to react to any stimulation, provided the stimulus is not at once
destructive.   This reaction ought to occur as the result of the
attack of an ultravirus, and to recognize its nature let us take the

simplest case, the initial reaction of the bacterium when parasitized by the bacteriophage.

Take two identical bacterial suspensions and inoculate one with a bacteriophage of maximum activity. Place both of the suspensions at 37°C. After two or three hours, it will be seen that the parasitized suspension is more turbid than the control suspension. Then, two or three hours later bacteriophagy takes place and the inoculated suspension will become limpid while the control suspension will continue to become more cloudy. The ultravirus, therefore, attacking the young bacteria, causes these bacteria to react immediately and to accelerate their processes of division before they are definitely overcome, and undergo lysis.

In different diseases the proliferative processes of cells under the excitation of the ultravirus is as readily demonstrable as in the case of bacteriophagy. In vaccinia, in variola, and in sheep-pox mitotic division of the attacked cells is augmented. The impression is obtained that the virus is leading to a neoplastic transformation (Borrel). In sheep-pox, the proliferation of the cells of the bronchial epithelium is so marked that actual adenomatous tumors form. In myxoma of the rabbit one witnesses the formation of cutaneous neoplasms (Sanarelli). In molluscum in man, in verruca, in epithelioma of birds, the lesions are formed entirely of epithelial neoplasms. The proliferative process reaches its maximum in sarcoma of the chicken where, under a stimulus provided by an ultravirus, the fusiform cells of the connective tissue divide in such an active manner that there is formed in a few days a very voluminous tumor (Rous).

This proliferation is also observed in cases where the attacked cell is a nervous cell. Although the latter does not proliferate itself (it seems incapable of doing this) it is none the less true that the neighboring cells undergo division. In rabies, Babès has shown that there is an accumulation of new-formed cells about the cells of the medulla and of the bulb, and at times a multiplication of the cells of the endothelial capsule. It is the same in canine plague, in which is to be seen a proliferation of the perivascular and periependymal neuroglia. In this last disease, there is a benign epithelial form, and here there is an intense proliferation of these cells.

The fact that in the majority of the diseases due to ultraviruses showing an elective affinity for nervous cells one observes a neuroglic proliferation, seems to indicate that these cells are the ones parasitized.

May it not be that the nervous cells are attacked secondarily? The ultravirus may first penetrate and multiply in the sheath of Schwann (which, as is known, is formed of a continuous syncytium) at the level of a peripheral nerve, reaching thus the nervous centres, and there parasitizing the nervous cells themselves.

In any case, the immediate reaction of the parasitized cell to the ultravirus is always a reaction of proliferation, provided the cell attacked is capable of effecting it, as is the case for all cells with the exception of the nervous cells.

THE CELLULAR LESIONS

There is no object to be gained by undertaking a histologic description of the lesions encountered in the different diseases caused by the ultraviruses.   We will simply mention the general lesions.

In bacteriophagy, following the initial stage of proliferation, a stage which is the more marked the lower the virulence of the ultravirus, *and which, in cases of extreme attenuation may be the only thing to take place*, comes the degenerative stage.   The bacteria attacked dissolve in the medium.

In the mosaics of plants, a necrosis also takes place with a fusion of the parasitized cells.

In the case of bacteriophagy, and even more in that of the mosaics, the ultravirus and the bacterium, or the vegetable cell, are the only two things present.   There is no interference by foreign elements entering to mask a part of the process of cellular destruction. On the contrary, when the ultravirus infects the cells of an organism as complex as a vertebrate, such interferences are necessarily produced by such things as the migratory cells, the leucocytes. In chicken sarcoma, this interference is indeed minimal.   Here the ultravirus acts exactly like a bacteriophage of weak virulence; only the proliferative stage takes place.[4]   In all other ultravirus

[4] The final stage of cellular disintegration in the case of the sarcoma does not appear to be due to the ultravirus, but must be caused solely by interference with the nutrition of the cell which, as a result of the ana-

diseases, the intervention occurs at the moment when the cell commences to undergo necrosis. There is an influx of leucocytes which engulf the débris. Can cellular lysis take place in the absence of the phagocytic reaction? It can only be know when we can apply methods of cultivation of the ultraviruses in cellular cultures in vitro. However, in variola, in vaccinia, and in sheeppox it is clearly seen that the fusion of the epithelial cells follows the initial proliferation.

In nervous cells the formation of vacuoles is always observed, and often a fusion, but here again, we do not know whether this fusion is really due to the action of the ultravirus. Here is, for example, the scheme of the lesions of poliomyelitis, according to the views of Landsteiner and Levaditi, and Marinesco:

    A phase of destruction of the nervous cell
    A phase of polynuclear attraction
    A phase of cytolysis
    A phase of neurophagy, that is to say, of elimination of the cellular débris by the macrophages

It is probable that the phase of cytolysis takes place under the action of the ultravirus, and not of the polynuclear cells, as was supposed by Marinesco, for the polynuclear cells never appear to attack the cellular débris.

However this may be, one is reduced to two conjectures because of the fact that the leucocytes interfere to mask the final stage of the infectious process. In any case, experiment shows that the ultravirus provokes a disorganization of the cell which involves the arrest of function and death. This "partial" death of the

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tomical structure of the tumor, is deprived of food. As for the absence of intervention by the leucocyte, it appears to be due to a negative chemotaxis between the leucocyte and the sarcomatous virus. Carré has observed that in the case of canine plague, the aggressive power of the leucocytes is found to be considerably diminished. This must occur with a number of ultraviruses, which may explain the invasion of the body by the "opportunist organisms" as is so often seen in the case of many ultravirus diseases. As for the attraction of the white cells, this attraction is exerted more by the presence of cellular products than by the presence of the ultravirus.

body parasitized, may involve the total death of the body by virtue of the complex inter-relationships of cellular processes.

The complete death takes place when the destroyed cells exercise an essential function, as is the case when the attacked cell is a nervous cell. The absorption of the products resulting from the cellular destruction may also play a part. Finally, the ultravirus as a living being which assimilates, necessarily pours out into its environment the products of its metabolism which may be toxic for the organism into which they are poured. It is impossible to actually understand the modes of action of these processes unless the mechanism can be determined, and at this time, the study of ultraviruses is not sufficiently thorough to determine these points.

THE CELLULAR INCLUSIONS

In almost all of the diseases caused by the ultraviruses, there have been observed in the cells attacked special bodies which have been the subject of many arguments. Negri was the first to note the presence of inclusions in the cells of the horn of Ammon in rabies. Guarnieri observed inclusions in the cornea in vaccinal keratitis of the rabbit. Prowazek has found these inclusions in the same cells at the beginning of trachoma. It is needless to review all of the inclusions which have been described, for there are but few ultravirus diseases in which they have not been found. Those who discovered them, naturally, wished to see a visible form of a parasite, but as a matter of fact such an opinion has found but few partisans, for it has been shown that these inclusions are always formed of a mixture of chromatin and of plastine, undoubtedly of nuclear origin. These inclusions represent a cellular lesion, that is all.

The ultraviruses are formed, beyond possible doubt, of protoplasmic micellae, and nothing warrants us in thinking for a moment that these micellae can form at any time a complex organism possessing an evolutionary cycle. All that could take place would be an aggregation of micellae, the formation of micellar plasmodia. Such a transitory aggregation is possible in the case of certain ultraviruses, and it is even probable in the case with those which are the most highly developed, that is, with those which approach most

nearly to the bacteria and the non-nucleated protozoa, but such a transitory aggregation can not be compared to an evolutionary cycle.

Furthermore, it is highly possible that the ultravirus may accidentally be found enclosed in the mass of chromatin and plastine which make up the cellular inclusion. I say "accidentally," for these inclusions are not constant in a given disease, as is exemplified by trachoma, where it is only by chance that one finds them in the first stage of the disease when the clinical diagnosis can not be established. Later they are never found, but it is "assumed" that they exist even though they can not be seen. Such a chance conclusion is, however, the less admissible since Bang has found comparable inclusions in cells which appear to be normal, without any virus infection, and even quite outside of all infectious disease whatsoever. As for the bodies of Guarnieri, they are found only in the vaccinal keratitis and not in the epithelial cells which are attacked. Negri bodies may be absent in rabies contracted after the inoculation of the fixed virus and several authors have sought in vain for them in the cells of rabid wolves.

From all of this one must conclude, and this is the generally accepted view, that the different inclusions described have nothing to do with the parasite itself. They result from a condition of toleration, they are the evidence of the cellular lesion, and are formed of materials derived from the nucleus of the cell attacked.

VACCINAL IMMUNITY

For a discussion of the processes of immunity in the diseases due to the ultraviruses we select two typical diseases, those most thoroughly studied, caused by organisms whose nature as ultraviruses has been determined by ultrafiltration, vaccinia and rabies. This choice offers the additional advantage in that in the first the epithelial cell is the one electively attacked and in the second the cell chiefly concerned is the nervous cell. If the processes of immunity which come into play are the same for both diseases we would have strong presumptive evidence in favor of the general nature of the reactionary processes against ultraviruses in general. And a comparison of that which happens in other diseases will show us that such a generalization is legitimate.

In the majority of the ultravirus diseases, it is impossible for us to follow the course of the cellular infection, but on the contrary, the vaccinal lesion, so readily produced, is accessible and it has been studied by many investigators.

With a minimal introduction of vaccinia virus, histological examination shows that first there takes place a traumatic reaction, leucocytic diapedesis and mitotic proliferation of the epithelial cells, comparable in all respects to that which occurs after an aseptic traumatism. At about the 48th hour the resemblance to the traumatic process ceases; the proliferation, far from stopping, increases and extends concentrically to the neighboring cells, progressively diminishing as it becomes more remote from the centre. At the centre of the zone of proliferation the cells vacuolize, become necrotic, and separate from each other. The interstices are invaded by migratory elements which, augmented by the débris of the cells which have succumbed form the vesico-pustule. Then the crust forms, which finally is detached, while the expelled tissue is replaced by a tissue formed by the surrounding cells, which, although they have undergone the proliferative excitation due to the virus, have nevertheless remained alive. The newly formed tissue comes then from cells which have resisted the attack of the ultravirus; from cells which have most probably succeeded in eliminating or in destroying the ultravirus.

The process is the same in the lesion which develops in the rabbit at the site where a hair bulb is removed, following the intravenous injection of the virus. In the case of direct vaccination many ultramicrobes are introduced, in the second case, the infection is certainly accomplished in the root by only a very few ultraviruses, possibly by only one. Nevertheless a pustule develops, and in intensity the lesion is the same.

How is it that the ultravirus which multiplies in the lesion, distributed from place to place from the centre to the periphery, causes at the centre a necrosis of the parasitized cells, while at the periphery there is only to be seen a simple proliferation of the cells which remain alive? The zone of multiplication of the ultravirus stops exactly at this barrier of the proliferating cells. These are then the cells which prevent its spread.

To comprehend just what takes place, let us consider once more

the bacteriophage. Take a very young agar culture of a bacterium, at a time when the layer of growth is not yet macroscopically visible. At a point on this layer deposit with a fine platinum wire a trace of the virulent culture of the bacteriophage. After incubation we will see that about the point inoculated with the ultravirus there is a clear area, where the bacteria have been destroyed. This area is, however, greater than the area inoculated and is proportional to the virulence of the bacteriophage used, at times reaching as much as 15 mm. Outside of the region where the infection has taken place the bacteria appear normal. If we remove the ultravirus which has developed at the expense of the bacteria which were within the area we will see that its virulence is as great as was that of the ultravirus inoculated. Around the area we can show that there is a ring of greater or less width where we may find the ultravirus which has progressed into the bacterial layer, and whose development has been less and less active the more remote it is from the central eroded area; very numerous at the inner edge of the ring, the ultramicrobes become more and more rare as we approach the outside. In addition, we can show that the virulence of these ultraviruses is markedly attenuated, just as these bacteria have a greater resistance to the action of the bacteriophage; this acquired resistance being related to the virulence of the ultravirus surrounding them. Outside of this ring there is no ultravirus and the bacteria are normal.

In connection with these facts I have likewise shown that the bacteria which have acquired a resistance to the bacteriophage destroy ultramicrobes of low virulence although they may be able to penetrate into their interior, a phenomenon which has been confirmed by the experiments of Flu. The defense of the bacterium against the bacteriophage is therefore double, it surrounds itself with a mucous capsule which impedes penetration, and if the bacteriophage succeeds in invading it, it may acquire the property of destroying the invader. This acquired resistance is transmitted through a number of generations, and is not completely lost until after from three to six hundred divisions.[5]

[5] Or, after 15 to 30 subcultures through isolated colonies. Calculation shows that, according to the conditions of the experiment, these transplants represent some 300 to 600 divisions.

All of these processes present a striking analogy to those which take place in the vaccinal pustule. At the centre all of the parasitized cells succumb and disintegrate while the ultravirus grows at their expense and spreads toward the periphery. At a certain distance the cells resist, and the attenuation of the virus follows the increased cellular resistance. Finally, the external portion of the ring which surrounds the vaccinal "plaque" is where the cells destroy the vaccinia virus which is already attenuated. As a matter of fact it is known that the virus of vaccinia, very virulent at the beginning, becomes weakened even to the point of being avirulent at the time when the central necrosed cells are eliminated by phagocytosis.

Here we might mention an accessory question. Experiment shows that all of the cutaneous covering acquires an immunity at the same time. It is probable that the attenuated ultravirus does not remain confined to the pustule, but passes into the circulation and is thus able to infect many cells of the skin. This contamination passes unobserved because of the low virulence of the germs which are quickly destroyed by the parasitized cells, as they most certainly are by the cells at the circumference of the pustule. Moreover, Levaditi has shown that whatever may be the mode of inoculation, cutaneous, intracerebral, or intratesticular, the virus becomes generalized very quickly, and is found after a very short time distributed throughout the organism.

But, you may say, is it thus legitimate to say that the cell possesses the property of destroying the vaccinia virus? Can not the immunity be humoral, can not the destruction of the ultravirus occur through the action of the blood, thus explaining readily and simply the fact of the simultaneous immunity of all of the cutaneous covering? This is a very important question, and deserves consideration.

When the vaccinal pustule dries up, there appears in the blood a specific "anti-virus" property. The serum neutralizes in vitro the vaccinia virus and it is of interest to know if this property is a primitive property of the blood or a consequence of the cellular immunity.

Camus immunized the rabbit against vaccinia, and bled the animals almost completely at several times. After each bleeding the blood of a normal animal was immediately introduced. He found

that the transfused blood acquired very quickly the "anti-virus" property to the same degree as in the original blood. The source of the neutralizing principle is then, not the blood itself, but the cells which discharge their products into the fluids.

A mother recently recovered from variola, or recently vaccinated, brings into the world a child who has an immunity, and its blood also possesses the anti-virus property. But while the blood of the mother retains its activity in this direction for a very considerable period, several months and sometimes even years, the blood of the infant loses it rapidly. It seems, then, that its persistence in the mother must be due to the fact that certain cells continue to elaborate the anti-virus principle, and that all that was present in the infant was a passive immunity.

When the blood of an individual possesses the anti-virus property this person has a solid immunity, but experiment demonstrates (Béclère Chambon and Ménard) that after some years, when this property has disappeared, the immunity still persists, as is shown by the fact that revaccination causes allergic reactions.

Recently Levaditi and Nicolau have disclosed a similar fact. When rabbits are vaccinated by the cutaneous route and tested after a variable length of time by an intracerebral injection of neuro-vaccine (that is, a vaccine which has become adapted to the nervous cells) they are resistant while the control animals die of vaccinal meningitis. At the same time the blood was taken and tested for its anti-virus property. They showed that a solid immunity persisted, even when the blood had lost all of its potency. The active principle is therefore produced by the cells.

During our consideration of antitoxic immunity we reached the conclusion that antitoxin could only be elaborated by the phagocytic cells of the endothelium of the blood capillaries and lymphatic vessels. Is the principle the same in the case of the ultraviruses?

Technical difficulties prevent us from proving whether the epithelial cell destroys the virus, but Levaditi has adapted the virus to the parasitism of the nervous cell. He obtained by means of a large number of passages through the brain of the rabbit a true "fixed" virus, which causes a fatal vaccinal meningitis. The virus multiplies in the brain, and is detectable there after the twenty-fourth hour and abundantly up to the time of death which

follows on the fifth day. But, if the virus is introduced into the brain of a previously vaccinated rabbit it can be shown that this virus is quickly destroyed, within two hours it can no longer be demonstrated and this destruction is accompanied by no cellular lesions whatever.

The experiments of Levaditi prove then, clearly, that it is the susceptible cells which acquire the immunity, and that this immunity is due to the fact that these cells are able to destroy the virus. The anti-virus property of the blood is secondary, the active principle is elaborated by the susceptible cell. We know that biological reactions are never in proportion to the excitation which provokes them. The cell continues to elaborate the active principle for a long time after the stimulus caused by the ultravirus has ceased. And this cell transmits this power to the daughter cells. The time during which this elaboration is produced is very different for differing animal species (for vaccinia, in the rabbit, some weeks, in man, sometimes for 15 years) and that it is even materially variable for individuals. We know in fact that certain men are not able to acquire vaccinal immunity, they react just like new subjects to all inoculations, however close together these inoculations may be. The elaboration of the active principle becomes less and less pronounced, and a time is reached when it becomes so weak that its presence can not be demonstrated in the blood. But the cell, nevertheless, retains for a long time the power to react.

We know, on the other hand, that a cell which has once reacted to a stimulus, reacts more strongly and more quickly to a second stimulus of the same nature. "It remembers" the reaction effected and here we must find the explanation of vaccinal allergy, as disclosed by von Pirquet. It is known that if we make a vaccination in a susceptible individual, the pustule forms only at about the fourth day. If a revaccination is given very soon after the first no general reaction follows. But as quickly as after two or three weeks, a local reaction takes place after but a few hours, and it develops more slowly as the immunity diminishes.

Moreover, I do not see how we can reconcile the different processes by combining them under the generic term of "allergy." The mere combination under a single term of differing phenomena,

which may have between them only a rather remote analogy (such as vaccinal allergy and allergy to tuberculin) is not sufficient reason for assuming that the mechanism is the same.

ANTIRABIC IMMUNITY

Rabies is a disease which is transmitted from animal to animal by the direct inoculation of the virus which is present in the saliva of the animal affected with the disease. This inoculation usually takes place through a bite, sometimes by licking or scratching. Rabies does not necessarily develop after the infecting bite, since the majority of cases, about 80 per cent, of men bitten by mad dogs remain uninfected. On the contrary, when bitten by a mad wolf, the disease occurs in 80 to 90 per cent of persons, either because the virus becomes more virulent by passage through the wolf, or because the bites are more severe. In any case this shows clearly that the virus may be destroyed in the body of the non-immunized individual. When the disease develops, the period of incubation has a minimum of about ten days, but usually it is between thirty and sixty days, sometimes even several months.

Pasteur showed that the passage of the virus through the rabbit (as also through the monkey) causes an adaptation for the nervous cells of this animal, and an attenuation for other animals, for man and the dog in particular. The subdural inoculation of a fragment of brain tissue from a mad dog kills the rabbit after a period varying between ten and sixteen days usually. As a result of the passage from rabbit to rabbit, the time of incubation is shortened and becomes regular. After about 100 passages the animal dies regularly in six to seven days after the inoculation. This virus of repeated passage is called "fixed virus," the virus as derived from the mad dog is termed "street virus."

A rather strange fact, noted by Fermi, which is in accord with what we have previously said concerning the variability of the ultraviruses, is the variability of the different fixed viruses. Since the work of Pasteur antirabic treatment has been provided by a number of laboratories, which preserve, by passages through rabbits, the fixed virus necessary for the inoculations. But, Fermi has seen that the fixed viruses of the Institutes at Bologna

and at Milan when injected subcutaneously into the rat failed to cause rabies, while that of the Institute at Florence caused a mortality of 36 per cent, those of Rome and of Turin a mortality of 60 per cent, and finally, those of the Institutes of Palermo and of Sassari caused rabies in all rats without exception. These fixed viruses of the different Institutes, although maintained by identical passages through rabbits, have not exactly the same properties. This variability does not, however, appear to have any influence upon the immunity conferred upon man or upon animals by the subcutaneous injection of the viruses.

After Pasteur had shown that the virus of rabies underwent an increase in virulence for the rabbit by repeated passages through the brain of these animals, and that there was a correlative attenuation for other species of animal, that there occurred, in a word, an adaptation of the virus for the infected cell, he discovered that if he slowly dried the cord of a rabbit dead of rabies the "activity" of the virus diminished gradually and became avirulent after about ten days. Pasteur advanced the hypothesis that what took place under the effects of the desiccation was not an attenuation of virulence, but a gradual destruction of the virus. In proof of this he showed that if he injected subdurally into a rabbit a suspension of cord which had undergone a slow desiccation for five days, the rabbit died only after about sixteen days, but if he made serial passages with the cord fresh from the last rabbit, *after a limited number of passages* the virus recovered its original activity. I think that this experiment shows, on the contrary, that there is an attenuation of virulence, and not a partial destruction of the virus, for in this last case, it is not after a series of passages that the virus should recuperate its original activity, but after the first passage. Just as soon as serial passages become necessary then there is involved an increase in virulence, which proves that there must have been an attenuation.

This attenuation takes place certainly under the action of cellular substances, for if the desiccation is rapid it does not occur and even when dried and in powder form the virus retains all of its activity for several months.

Pasteur showed that, in the dog, a series of subcutaneous injections of emulsions of the cords of rabbits killed by the fixed

virus, prepared from cords more and more virulent, that is to say, less and less dried, commencing the series with an emulsion of a cord dried for fourteen days, and ending with an emulsion of cord dried for three days, conferred an immunity. And he applied this treatment to man during the incubation period of rabies. In man the incubation period after the bite is usually more than thirty days. The virus travels slowly from the point of inoculation up to the nervous centres, most probably following a nerve, but just as quickly as it gets to the nervous centres the disease develops with rapidity. The problem consists then in taking advantage of the incubation period and in such a way that the attenuated virus reaches the region of multiplication, that is, the brain, before the arrival of the more virulent virus. The latter then finds the cells vaccinated, it is not able to parasitize them, and the disease does not take place.[6] This is, in effect, the method applied in the Pasteur treatment.

What is the nature of the immunity against rabies?

The nervous cells appear to be the only ones at the expense of which the rabies virus is able to grow. The other species of cells, instead of lending themselves to culture, destroy the virus. Of this we have numerous proofs. It is known that rabies does not inevitably occur, neither in man nor in animals, after the bite, and that subcutaneous injections of the virus give but inconstant results. It seems, therefore, that in the majority of cases, the virus must be destroyed before it gains access to the nervous tissues. In the dog, the disease can not be transmitted serially by subcutaneous injections. After two or three passages the virus is no longer infective for this animal. With the herbivora, disease never takes place after intravenous injection and the animal is vaccinated (Nocard and Roux). In the dog, the rabbit, and the guinea-pig, the intraperitoneal injection is inoffensive, and it can be shown that the virus is quickly destroyed in the peritoneal cavity, although we do not know by what mechanism, for, in vitro, the peritoneal exudate does not destroy the virus.

In 1891 Babès showed that the sera of immunized animals possessed the property of neutralizing, in vitro, the rabies virus,

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[6] At least this is the explanation generally advanced.

either fixed or street virus. Let us state that this property has
nothing to do with the assumed "bacteriolytic" property of sera,
for the neutralization is effected, as in the case of the antitoxins,
without the intervention of complement. Old sera, sera heated
to 60°, possess an action exactly comparable to that of fresh serum.
The antivirulin resists temperatures even as high as 72°. This
remark does not apply solely to the anti-rabies serum, but to all
sera possessing the property of neutralizing an ultravirus, whether
this virus is that of bacteriophagy, of vaccinia or that of one of the
animal plagues.

The serum of an immunized animal, mixed in vitro with some
filtered rabies virus neutralizes the virus, for the injection of such
a mixture under the dura of a susceptible animal never causes
rabies. Is the virus destroyed or simply rendered avirulent? As
we have seen, it is impossible to distinguish between loss of viru-
lence and destruction when we are dealing with an ultravirus. Yet
in the case of the bacteriophagous ultravirus we have given the
proof that it is a loss of virulence, and it is probable that it is the
same for all ultraviruses.

The active substance fixes itself to the virus, but the complex is
easily dissociated. A mixture of anti-serum and of rabies emul-
sion is centrifuged and the sediment which is collected is washed
with saline. This saline when inoculated causes rabies in a sus-
ceptible animal.

The active substance fixes itself to the ultravirus, for if one
centrifuges a neutral mixture of the emulsion of rabic cord and of
the antiserum, one finds that the supernatant serum has lost all
of its antivirus property. The active principle is then fixed to
the virus. It might be objected that it is perhaps the cerebral
substance which fixed the antivirulin, but if, in the preceding
experiment, the emulsion of rabies cord is replaced by an emulsion
of normal cord, fixation does not take place, indicating beyond
doubt that it is really the ultravirus which does the fixing.

Observe a rather interesting fact, namely, that for sensitization
to be effected it is necessary for the serum and the virulent cord
to be in suitable proportions. An excess of serum interferes with
the neutralization.

An anti-rabies serum of maximum activity is capable of

neutralizing 40 times its volume of an hundredfold dilution of the the cord of a rabbit killed by the fixed virus. This does not indicate, however, the true activity of such a serum, for the ultravirus is somewhat protected by the nervous tissue in which it is embedded. This is shown clearly in that if the neutralization is effected with an emulsion of cord previously filtered through paper in order to eliminate the largest fragments, it does not act like an unfiltered emulsion. To say that a serum neutralizes so many volumes of an emulsion of rabies cord, means absolutely nothing as to its true activity, and such a measure is of only comparative interest.

Is the neutralizing property due to the presence in the antiserum of a substance or is it a property, a special colloidal state, as is the case for that which we call alexin? It is certainly a substance, for the anti-property is stable and withstands changes in the equilibrium of the serum. It is not destroyed by aging nor by heating at 72° although this profoundly modifies the state of equilibrium of the serum. This principle has not yet been provided with a name, and for it I might suggest the term antivirulin, and the serum which contains it might be termed an antivirulic serum.

We have seen that the place of formation of the vaccinal antivirulin must be the susceptible cell itself. Is it the same for the rabies antivirulin?

We know that in tetanus the nervous cell is not vaccinated even though the blood of the animal carries an enormous quantity of antitoxin, since the intracerebral injection of a minimal quantity of toxin kills such an animal as readily as it does a normal animal. This is not the case in rabies. Pasteur was the first to show that a dog thoroughly vaccinated resisted the subdural inoculation of the virus. On the other hand, the immunity of all of the susceptible cells, of all of the nervous cells, is not equal. A number of vaccinated animals succumb to a subdural inoculation but they resist an intra-ocular injection, indicating that the peripheral neurones acquire the immunity more readily or more quickly than do the nervous centres.

This cellular immunity is enduring. Pasteur has shown that certain vaccinated dogs could resist two years later a subdural inoculation.

Marie, by treating the brains of immunized animals by repeated precipitations with acetic acid, has been able to isolate a product possessing the property of neutralizing the virus in the same manner as the antiserum.   The brains of normal subjects also contained the same principle, but of much less activity; if the substance from the normal brain be given a value of 1, the same substance derived from the brain of the animal dead of rabies had a value of 2, and that procured from the brain of the immunized animal had a value of 20.

This experiment shows that antivirulin is elaborated by the sensitive cell, the nervous cell, and that it is poured into the blood, as is the case in vaccinia.

We see that antivirulin exists in the brain of the normal animal, and because of this fact the animal should have some natural immunity to rabies.   We know that as a matter of fact such an immunity exists, for all persons bitten do not contract the disease, and this is as true for man as for dogs or other animals.   We have seen further, that the subcutaneous inoculation is not able to overcome the resistance of an animal in the majority of cases. As we have seen above, many authors have attributed this fact of non-infection after subcutaneous inoculation to an attenuation or a destruction of the virus in tissues other than the nervous tissue. I do not think that this explanation is true.   The disease fails to take place because the animal possesses a natural immunity of greater or less degree.   The following shows that this must be the real reason.

Certain animals, belonging to susceptible species, rabbits for example, enjoy *naturally* an immunity of such a degree that they may resist subdural inoculation (Remlinger).   In addition it is known that the virus is able to live for several months in a latent state in the brain, the disease only becoming manifest as a result of the diminution of the resistance of the organism.   Such animals are therefore endowed with a certain immunity.   This indicates that all animals enjoy a certain degree of natural immunity, and that when contaminated, those which possess it to a weak degree contract the disease, and those in which it is more highly developed resist, provided some intercurrent cause does not intervene to bring about a transitory weakening.

We have seen that in variola and in vaccina, there is a certain transitory immunity transmitted from the mother to the offspring. In rabies it is the same, but here the inherited immunity may be of longer duration. Konradi has seen it present in rabbits as long as two years and two months after birth.

In summary, then, all of these things show that, as in vaccinia, the immunity against rabies, in susceptible animals, is of cellular origin. The cell reacts to a stimulation caused by the ultravirus by the elaboration of a substance, an antivirulin, which has the property of inactivating the ultravirus, and of which the excess is poured out into the blood.

IMMUNITY IN DIFFERENT DISEASES

In many diseases caused by ultraviruses, it has been possible to demonstrate the presence in the humors of immune animals, of a principle possessing the power of specifically inactivating the ultravirus, the cause of the disease. Such is the case for the plagues, avian, bovine, swine, canine, for sheep-pox, for poliomyelitis, and for measles.

As yet there are but few experiments relative to the origin of these antivirulins, but the few facts known show us that, as in the case of rabies and of vaccinia, they must be derived from the cells which are parasitized.

In herpes, neither Levaditi and Harvier, nor Blanc, have as yet been able to disclose such an inactivating principle in the fluids, but on the other hand, Levaditi has demonstrated that such a principle exists in the brains of immune rabbits.

It is then extremely probable that it is a general fact. The cell reacts against the excitation caused by an ultravirus by the production of a specific antivirulin.

NATURAL IMMUNITY AGAINST ULTRAVIRUSES

With regard to the bacterial diseases, it was stated that there is absolutely no a priori right to group a natural species immunity, that is to say, the refractory state which occurs in those animals belonging to a given species for a given bacterium, with the immunity acquired by an animal belonging to a susceptible

species. The mechanisms of the two immunities must be very distinct.

And it is for this reason that all experiments dealing with acquired immunity, effected upon refractory animals, have no value whatever. The same remark applies equally well to diseases caused by ultraviruses, and here the proof that the mechanism of natural immunity differs from that of acquired immunity may be experimentally furnished.

Birds are refractory to rabies. The blood of the adult pigeon does not manifest the property of inactivating the rabies virus, yet this animal is completely refractory to rabies; even intracerebral inoculation does not cause the disease, yet the virus is not destroyed in the brain (as is the case for sensitive animals with an acquired immunity), and the virus is still present after twenty-five days. When inoculated into rabbits it causes rabies.

Repeated injections of virulent cord into a susceptible animal reinforce the immunity. Repeated injections into the pigeon do not lead to the appearance of any antivirulic property, either in the brain or in the fluids. It is the same for the goose.

From this it appears that while immunity against rabies in the susceptible animal is due to the elaboration by the sensitive cells of an inactivating principle, immunity in the refractory pigeon and in the goose is due to a complete lack of an affinity between the virus and the nervous cells. The cell escapes parasitism. The mechanism of this cellular immunity is identical with the *natural* immunity of bacteria to the bacteriophage.

These experiments confirm further that the antivirulin is the result of a specific reaction of the attacked cells, since, when the cells can not be attacked there is no formation of antivirulin.

EXPERIMENTALLY ACQUIRED IMMUNITY

It is needless to dwell upon the discovery of Jenner, which has had such an important influence upon the development of our ideas and upon the study of the infectious diseases. The fact of immunization against variola, which was up to this time the most terrible of the infectious diseases, by the inoculation of the virus of cow-pox, is such a certainty that it is necessary to willfully close the eyes to have any doubt of it.

Nor will we dwell upon the discovery of Pasteur regarding immunization against rabies, the mechanism of which we have been considering. While for the antivariola vaccination Jenner made use of a virus attenuated for man through the operation of a natural adaptation to parasitism of the susceptible cells of the cow, Pasteur accomplished the same attenuation by an experimental adaptation of the rabies virus to the susceptible cell of the rabbit. With Jenner we are concerned with a process accomplished by nature, with Pasteur we deal with an accidental phenomenon, which we can accomplish ourselves.

Högyes has modified the method of Pasteur. In the place of the attenuation of the virus adapted to the rabbit, of the fixed virus, by desiccation and aging of the cords, he utilizes dilutions of fresh cord, practising a series of inoculations with emulsions less and less diluted. However, the two methods give identical results.

Doubtless these methods could be modified. The fixed virus actually employed in the different Institutes has undergone since Pasteur many thousands of rabbit passages. The adaptation of the virus to the rabbit is accentuated and one of the proofs of this is that it is possible to inoculate man with large quantities of fresh cord without great danger, and without the need of preparing him first by inoculations of aged cords. With the real fixed virus there would be the advantage of modifying the original treatment (which Pasteur based upon the use of a fixed virus having but a limited number of rabbit passages) and of inoculating at once, if not fresh cord, at least cord which has been desiccated for five days in the place of the cord dried for fourteen days as is the custom.

For several centuries before the Jennerian era Oriental peoples, and then European, practised variolization, that is, they inoculated the infant whom they wished to protect against variola with a powder prepared by grinding up the crusts removed from variola pustules. They selected for this purpose cases with a relatively benign variola. The method was not without danger, and involved a considerable mortality. The same method has also been employed for the immunization of sheep against sheep-pox, the procedure causing in this animal a mild attack due to the inoculation of the lymph derived from a sheep-pox pustule. The

injection is given upon the internal surface of the ear or in the region of the tail. Here again the method is attended by danger, and the mortality at times reaches as high as 10 per cent of the animals vaccinated.

Borrel has shown that if one injects at several different times a sheep recovered from sheep-pox with the lymph removed from the pustules of sick animals, the blood of the animal possesses both curative and preventive properties. But, since it is a passive immunity it is of short duration. Bridré and Bocquet have conferred a much more durable immunity (five months at least) by employing the virus-serum method indicated by Kolle and Turner for bovine plague. After the injection of the sheep-pox pulp which had been in contact with the anti-sheep-pox serum the animal showed a febrile reaction and a transitory edema at the point of inoculation. The animal then resisted, just as after a natural attack, the experimental inoculation of large amounts of virulent lymph.

This method of immunization, which, as stated, was first used by Kolle and Turner, can be employed in both bovine and swine plagues.

We might note that this confirms that which experimentation with the antibacteriophage serum has shown, namely, that an anti-serum does not destroy the ultravirus, but causes a simple inactivation more or less marked.

ANTIVIRUS IMMUNITY

Antivirus immunity is extremely powerful. For a long time simple observation has shown that relapses are rare in those diseases which we actually know to be caused by ultraviruses. One attack of such a disease, aside from infrequent exceptions, renders the individual refractory for the remainder of his life.

Experiment has confirmed the fact of the firmness of antivirus immunity. With regard, for example, to bovine plague it is possible to inject into a recovered animal several liters of virulent exudate without causing the least disturbance, when the injection of a trace of this exudate into a normal animal would lead to a fatal infection. Comparable facts have been disclosed for a number of ultravirus diseases.

All of the facts show us that this immunity is of cellular origin. The parasitized susceptible cell reacts to the presence of the ultravirus by the production of a principle which possesses the property of inactivating specifically the stimulating ultravirus.

Because of their stability these inactivating principles must be, not properties resulting from an equilibrium, but substances, analogous to, but not identical with, the antitoxins.

During a certain time after the susceptible cell has reacted to the excitation caused by an ultravirus through the elaboration of an antivirulin the antivirulin is produced in excess and is eliminated from the cell, passing into the blood where it can be demonstrated. But for a long time after this elimination has ceased, the cell retains its property of inactivating the ultravirus to which it at one time reacted, and this immunity is transmitted through heredity from the maternal cell to the daughter cells.[7]

The immunity against the ultraviruses is a special immunity, which is related to antitoxic immunity. In all essentials it is different from the endogenous antibacterial immunity solely of the phagocytic order.

CONCLUSIONS

The unity of specific reactions

Up to the present time we have considered the problem of etiology, together with that of pathology, of the infectious diseases as being relatively simple. It has all been reduced to a study of the mutual reactions between the parasitic bacterium and the susceptible animal. But the problem is far more complicated than at first appeared. In the "transmissible" infectious diseases three factors are involved—the susceptible organism, the parasitic bacterium, and the bacteriophagous parasite of the bacterium—and each of these three factors reacts to the other two. In the "contagious" infectious diseases, that is to say, in the epidemic diseases, a fourth being may be introduced, sometimes an insect, the inoculator, and often an ultravirus, an agent of contagion.

These facts will not be reviewed, for they have been treated at

[7] This is not the equivalent of saying that it is an immunity that is transmitted to the descendants of the individual.

some length already, but there is one further point which should be emphasized in closing this discussion.

Present-day immunology, based upon fictitious experiments, and upon which have been constructed the complex theories which have nothing to do with the facts, singularly complicate the mode of reaction of the infected organism. If it represents the problem of etiology as a simple process it compensates for this by the complexity with which it describes the process of reaction. And in reality the reactional process is the simple thing.

In accord with these theories when a bacterium invades the body the body reacts. And it reacts by the production of a large number of principles which lead to diverse phenomena—agglutination, precipitation, complement fixation, bactericidal action, bacteriolysis, opsonic action, and anaphylactic sensitization. In reality it is quite otherwise. There is but a single "antibody," sensitizin, which leads to but a single phenomenon, a flocculation. All of the lytic phenomena which have been described are imaginary phenomena.

To a stimulation living matter always reacts, and the reaction is effected according to a scheme which harmonizes with the excitation. What is the essence of this rhythm? As yet we do not know, we only know that the mode of reaction of living matter is "specific."

There is but one specific reaction. Whether it be the endothelial cell reacting to the stimulus provided by the toxin micella, whether it be the endothelial cell reacting to a protein micella either living or inanimate, whether it be the epithelial cell or the nervous cell reacting to a pathogenic ultravirus, or whether it be the bacterium reacting to the excitation caused by the bacteriophage, the specific reaction is in all cases the same. There is in all cases an inactivation of the exciting principle through the intermediary of a flocculating principle elaborated by the cell.

The response of living matter is single, not multiple, and whether the product of this response be termed sensitizin, antitoxin, or antivirulin, it is always a "flocculin."

And what are the results of this single mode of reaction? They are of two types, and of very different types. One type tends

toward the conservation of the instable equilibrium, which means life, the other tends toward the rupture of this equilibrium.

The specific reaction is a physico-chemical reaction. It could not be anything else, for it is itself derived from the constitution of living matter. Its aim is not particularly the conservation of life although life may be a result of the reaction. But it is not strange that such a physico-chemical process expressing itself by a series of phenomena should manifest some reactions, which, from our human point of view, we choose to consider as acts of protection, of "prophylaxis," and others as acts of contra-protection, of "anaphylaxis."

This same phrase, also to be found in the preface of this text, may have appeared to many readers to be very venturesome. I trust that the facts recorded may serve as justification.

May I further add that I hope, indeed, I am convinced, that this method of viewing the phenomena and of approaching their study without preconceived ideas is the only method which will lead us to a recognition of the "nature of things" and a knowledge of the truth.

Sans Tache

Sans Tache

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